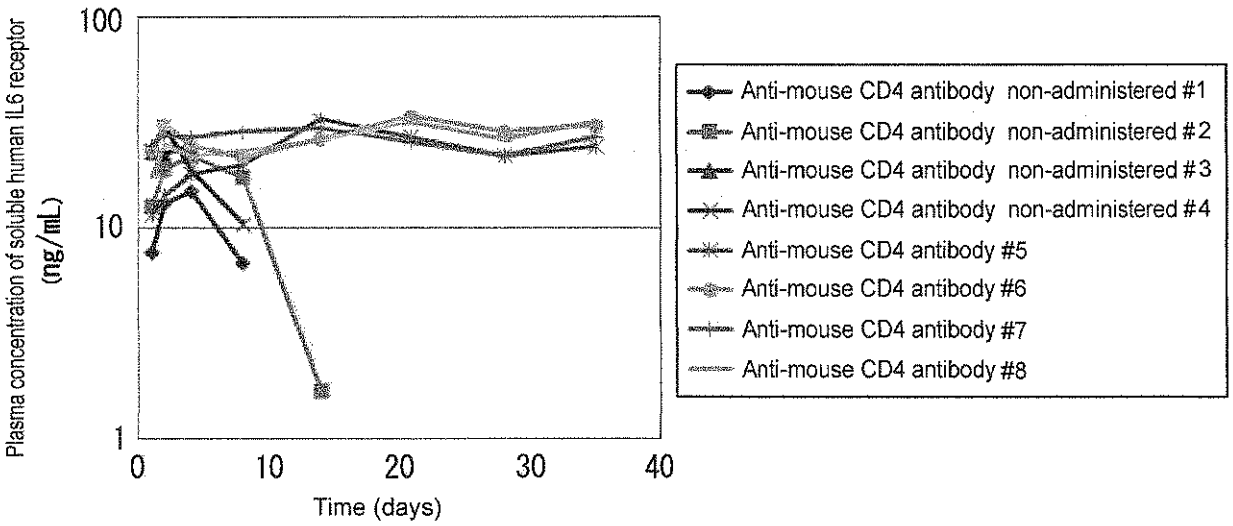

Plasma concentration of soluble human IL6 receptor (ng/mL)
100
10
1
0
10
20
30
40
Time (days)
Anti-mouse CD4 antibody non-administered #1
Anti-mouse CD4 antibody non-administered #2
Anti-mouse CD4 antibody non-administered #3
Anti-mouse CD4 antibody non-administered #4
Anti-mouse CD4 antibody #5
Anti-mouse CD4 antibody #6
Anti-mouse CD4 antibody #7
Anti-mouse CD4 antibody #8

FIG. 1

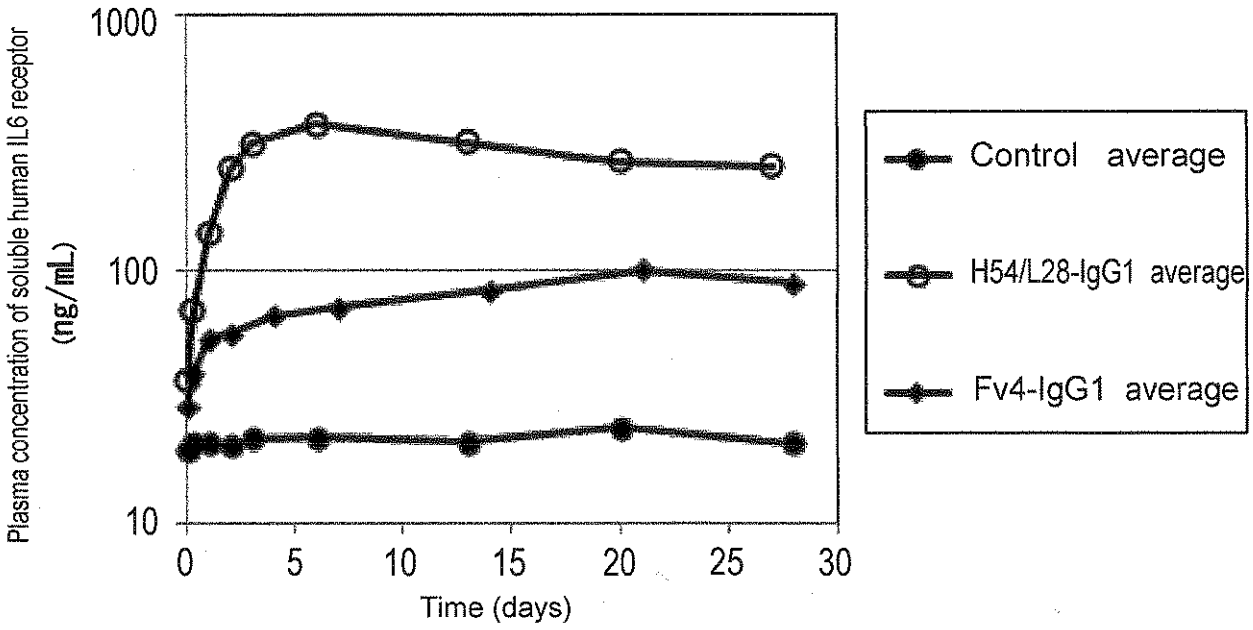

Plasma concentration of soluble human IL6 receptor (ng/mL)
1000
100
10
0
5
10
15
20
25
30
Time (days)
Control average
H54/L28-IgG1 average
Fv4-IgG1 average

FIG. 2

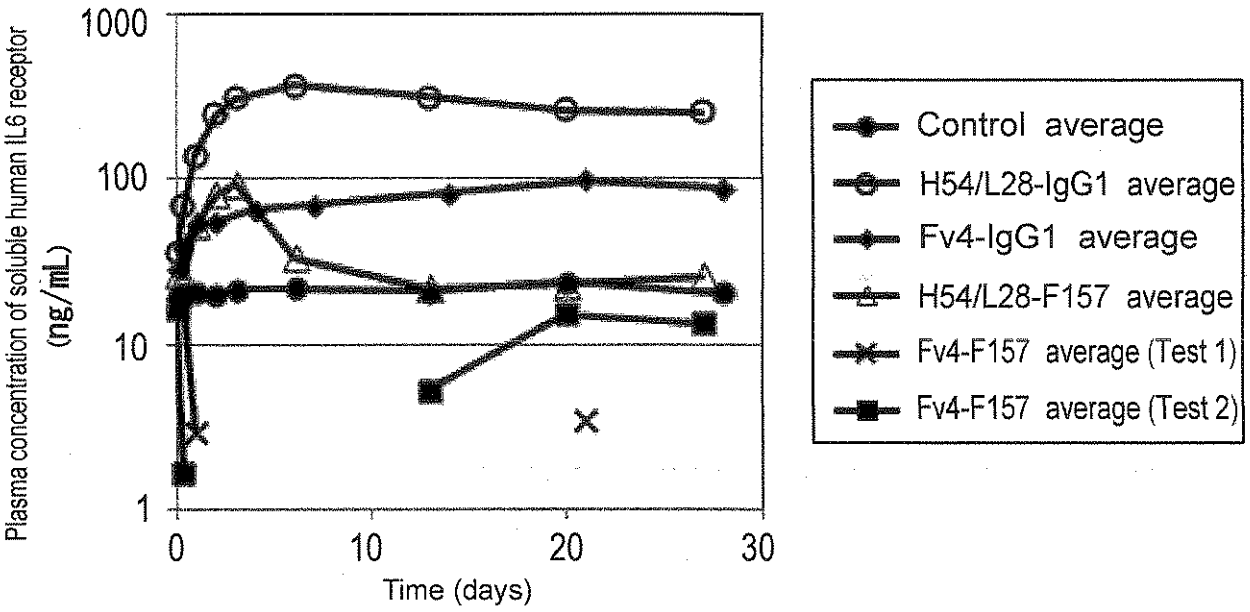

Plasma concentration of soluble human IL6 receptor (ng/mL)
1000
100
10
1
0
10
20
30
Time (days)
Control average
H54/L28-IgG1 average
Fv4-IgG1 average
H54/L28-F157 average
Fv4-F157 average (Test 1)
Fv4-F157 average (Test 2)

FIG. 3

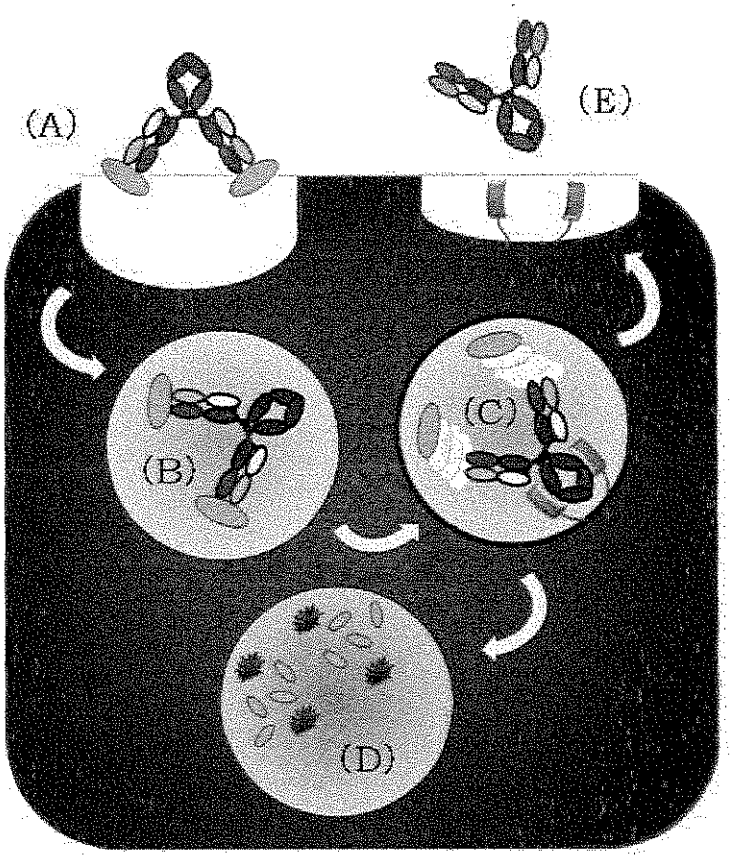


FIG. 4

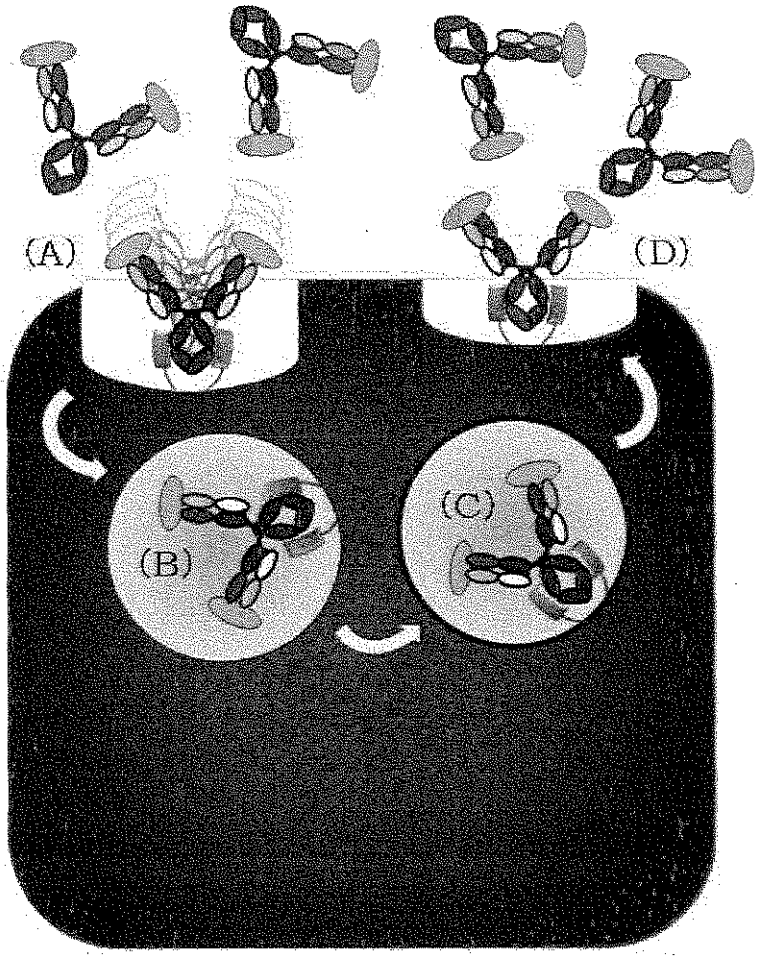


FIG. 5

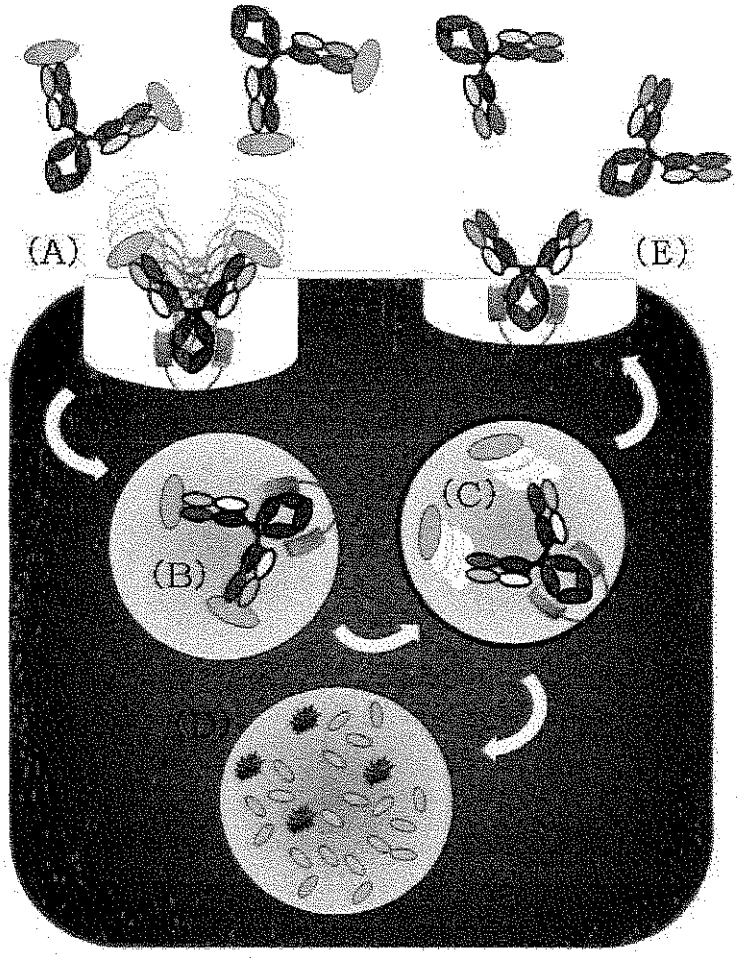


FIG. 6

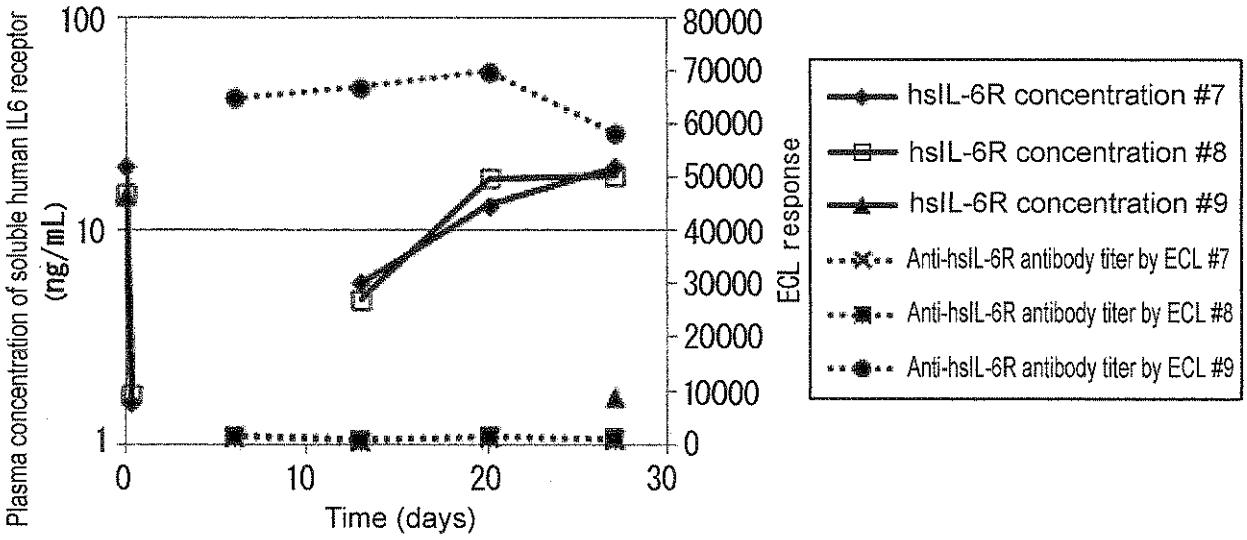

Plasma concentration of soluble human IL6 receptor (ng/mL)
100
10
1
0
10
20
30
Time (days)
80000
70000
60000
50000
40000
30000
20000
10000
0
ECL response
hsIL-6R concentration #7
hsIL-6R concentration #8
hsIL-6R concentration #9
Anti-hsIL-6R antibody titer by ECL #7
Anti-hsIL-6R antibody titer by ECL #8
Anti-hsIL-6R antibody titer by ECL #9

FIG. 7

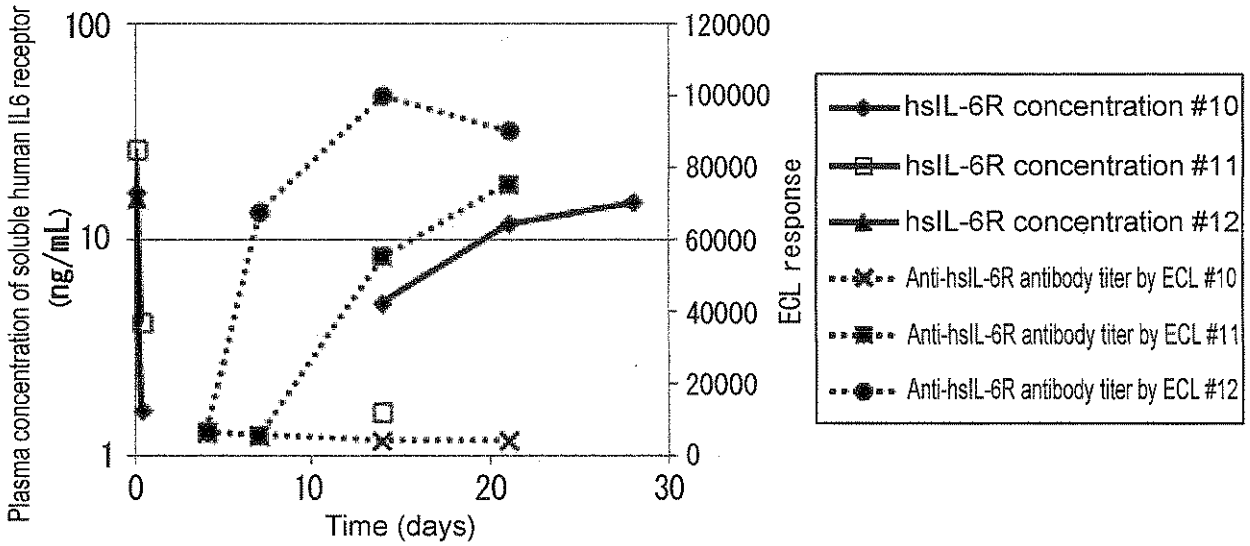

Plasma concentration of soluble human IL6 receptor (ng/mL)
100
10
1
0
10
20
30
Time (days)
120000
100000
80000
60000
40000
20000
0
ECL response
hsIL-6R concentration #10
hsIL-6R concentration #11
hsIL-6R concentration #12
Anti-hsIL-6R antibody titer by ECL #10
Anti-hsIL-6R antibody titer by ECL #11
Anti-hsIL-6R antibody titer by ECL #12

FIG. 8

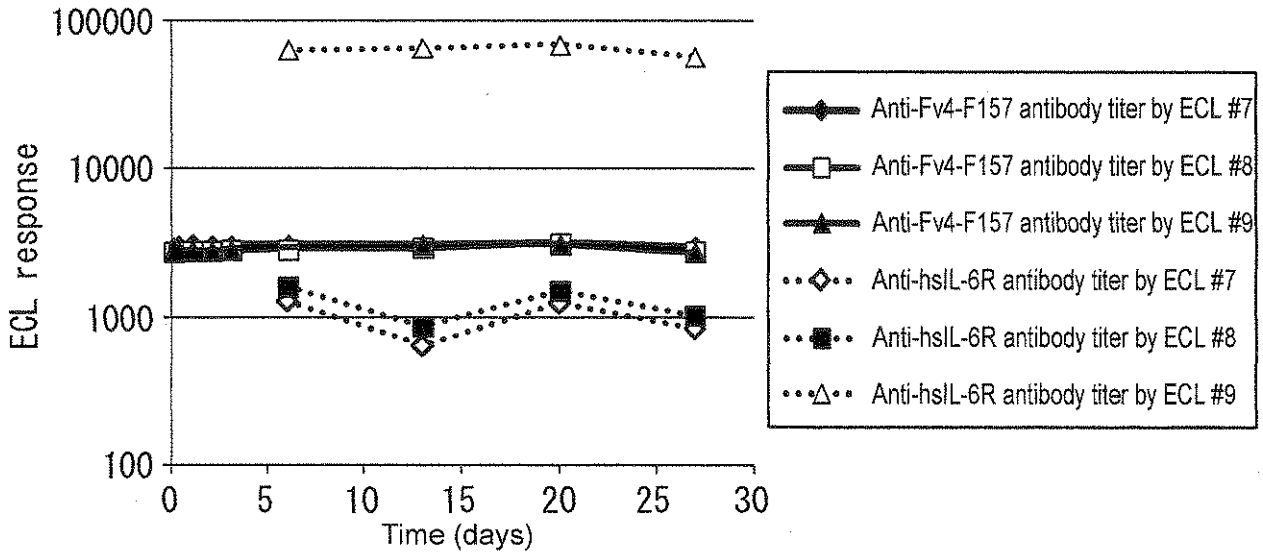

100000
10000
1000
100
ECL response
0
5
10
15
20
25
30
Time (days)
Anti-Fv4-F157 antibody titer by ECL #7
Anti-Fv4-F157 antibody titer by ECL #8
Anti-Fv4-F157 antibody titer by ECL #9
Anti-hsIL-6R antibody titer by ECL #7
Anti-hsIL-6R antibody titer by ECL #8
Anti-hsIL-6R antibody titer by ECL #9

FIG. 9

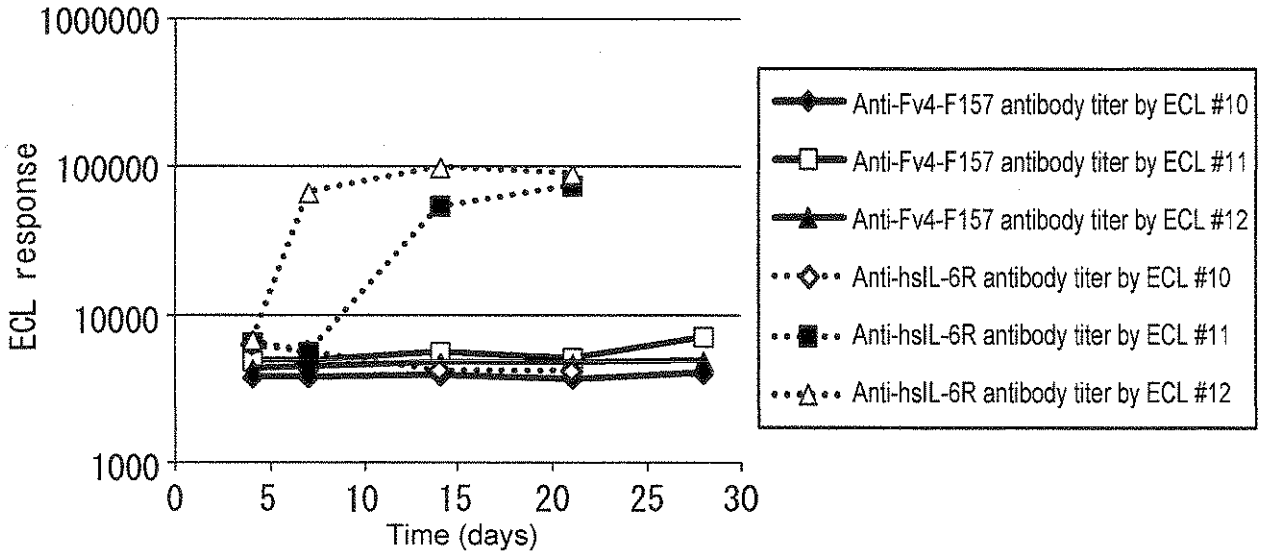

1000000
100000
10000
1000
ECL response
0
5
10
15
20
25
30
Time (days)
Anti-Fv4-F157 antibody titer by ECL #10
Anti-Fv4-F157 antibody titer by ECL #11
Anti-Fv4-F157 antibody titer by ECL #12
Anti-hsIL-6R antibody titer by ECL #10
Anti-hsIL-6R antibody titer by ECL #11
Anti-hsIL-6R antibody titer by ECL #12

FIG. 10

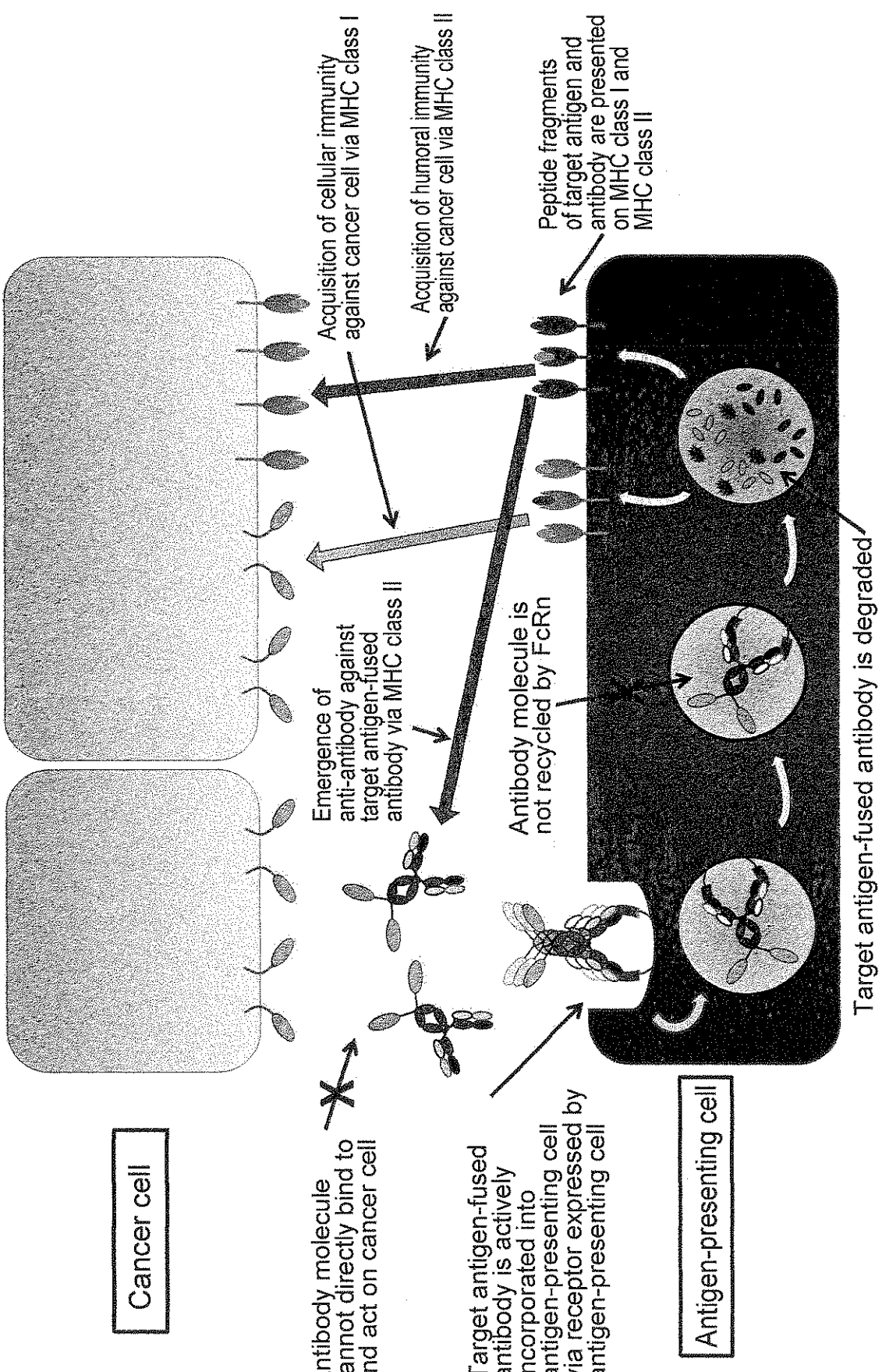

Cancer cell
Antibody molecule cannot directly bind to and act on cancer cell
Target antigen-fused antibody is actively incorporated into antigen-presenting cell via receptor expressed by antigen-presenting cell
Antigen-presenting cell
Emergence of anti-antibody against target antigen-fused antibody via MHC class II
Antibody molecule is not recycled by FcRn
Acquisition of cellular immunity against cancer cell via MHC class I
Acquisition of humoral immunity against cancer cell via MHC class II
Peptide fragments of target antigen and antibody are presented on MHC class I and MHC class II
Target antigen-fused antibody is degraded

FIG. 11

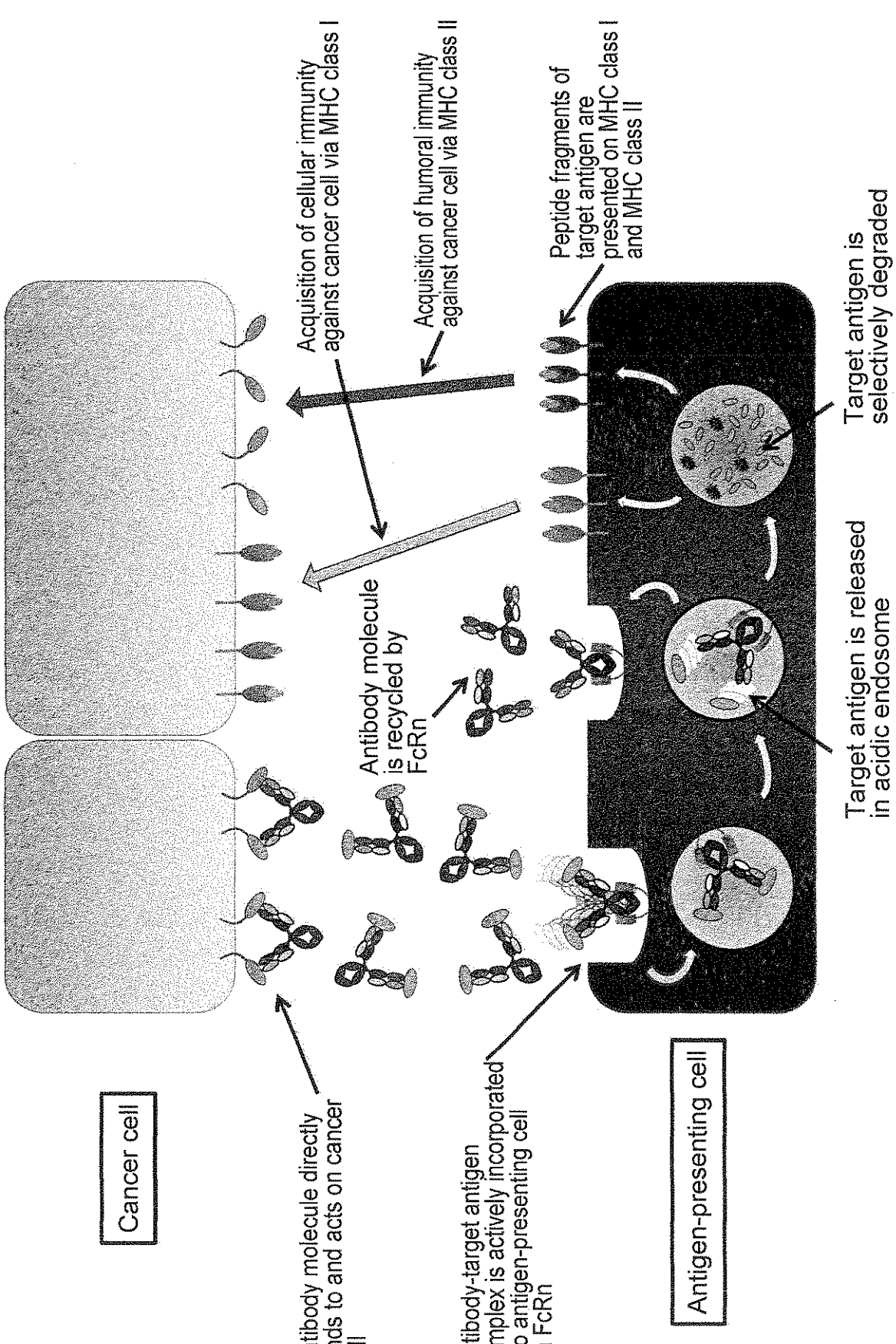

Cancer cell
Antibody molecule directly binds to and acts on cancer cell
Antibody-target antigen complex is actively incorporated into antigen-presenting cell via FcRn
Antibody molecule is recycled by FcRn
Acquisition of cellular immunity against cancer cell via MHC class I
Acquisition of humoral immunity against cancer cell via MHC class II
Peptide fragments of target antigen are presented on MHC class I and MHC class II
Target antigen is selectively degraded
Target antigen is released in acidic endosome
Antigen-presenting cell

FIG. 12

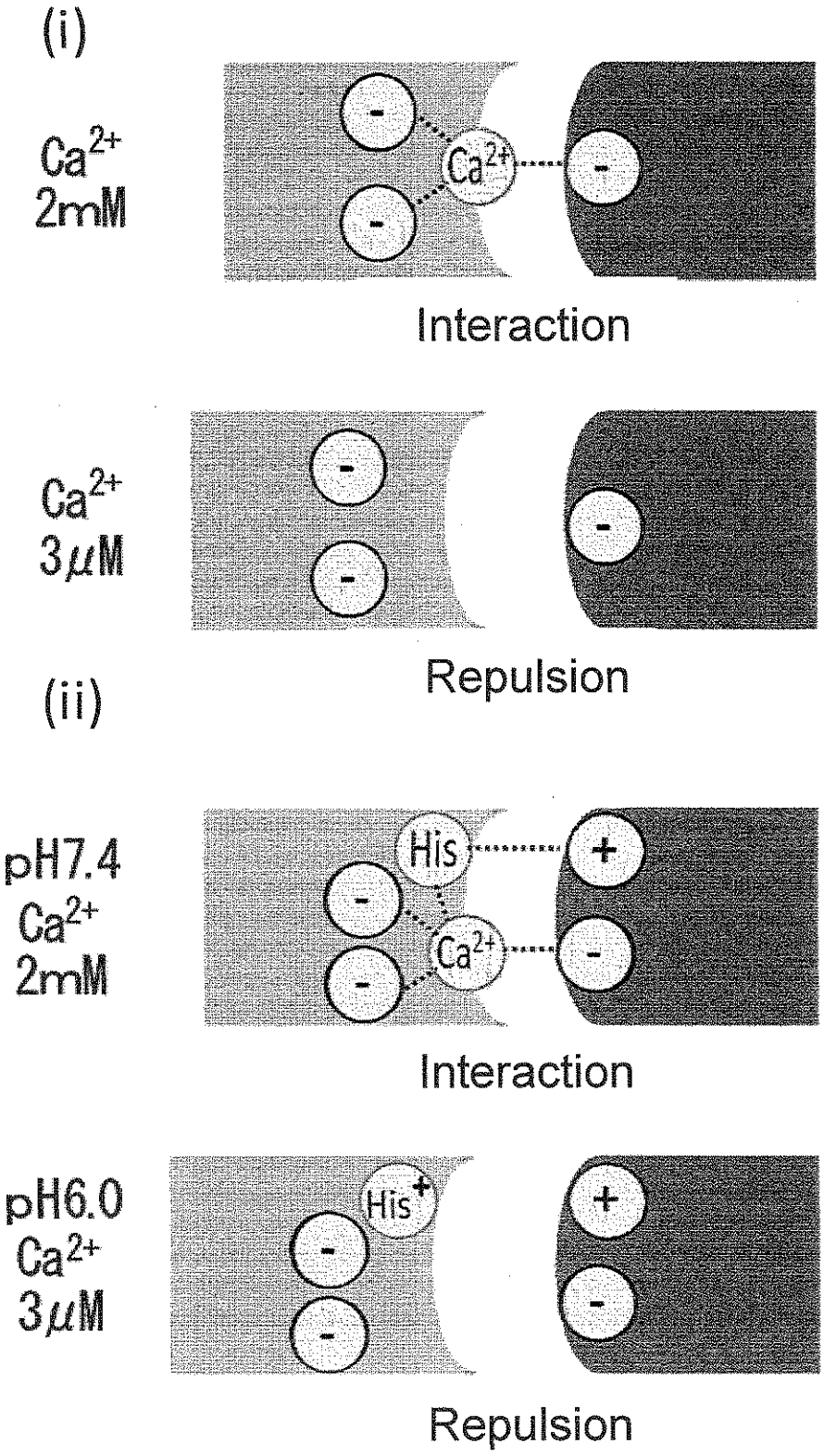

(i)
Ca^{2+}
2mM
Ca^{2+}
Interaction
Ca^{2+}
3μM
Repulsion
(ii)
pH7.4
Ca^{2+}
2mM
His
Ca^{2+}
Interaction
pH6.0
Ca^{2+}
3μM
His^+
Repulsion

FIG. 13

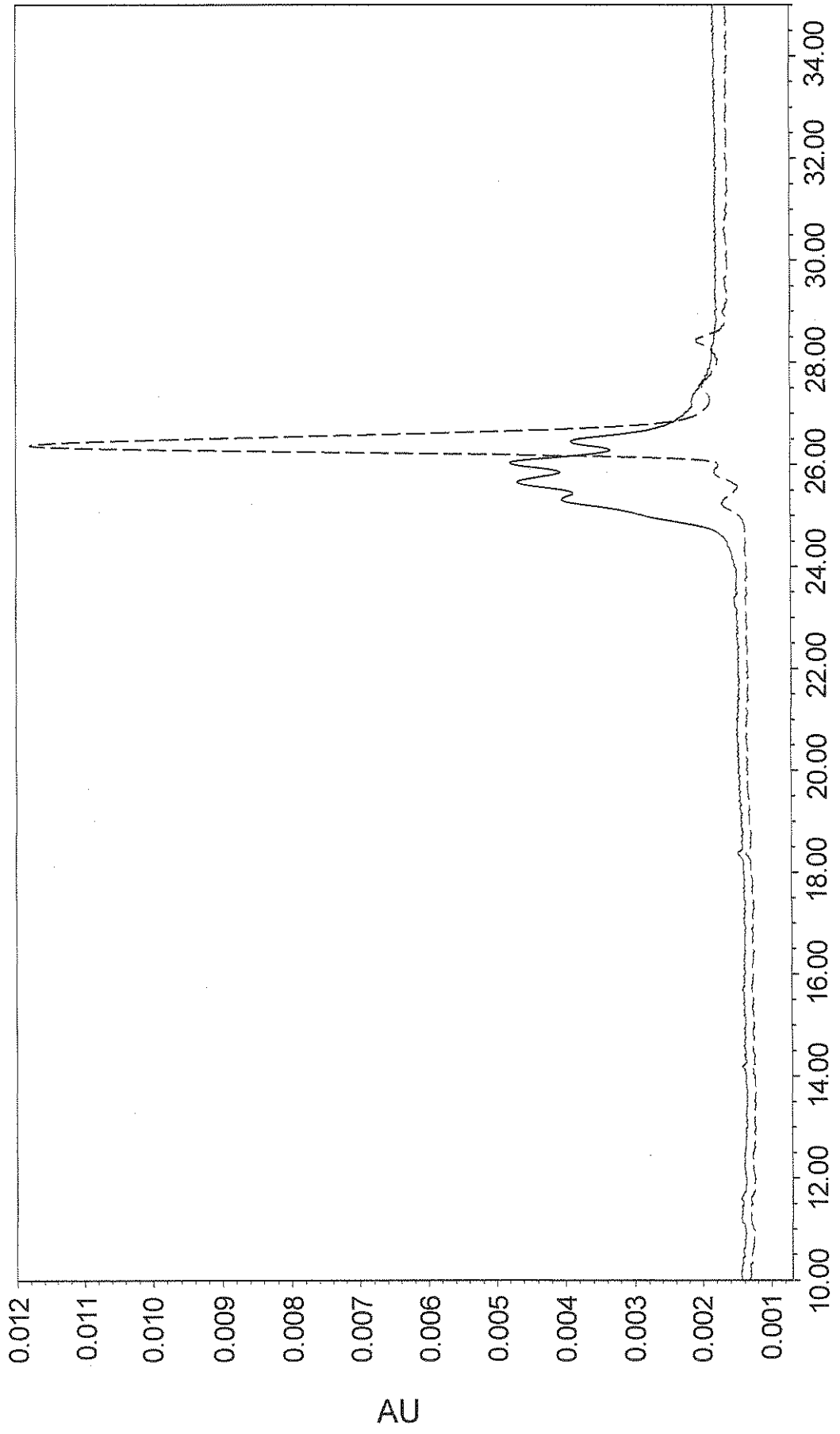

0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
AU
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
minutes

FIG. 14

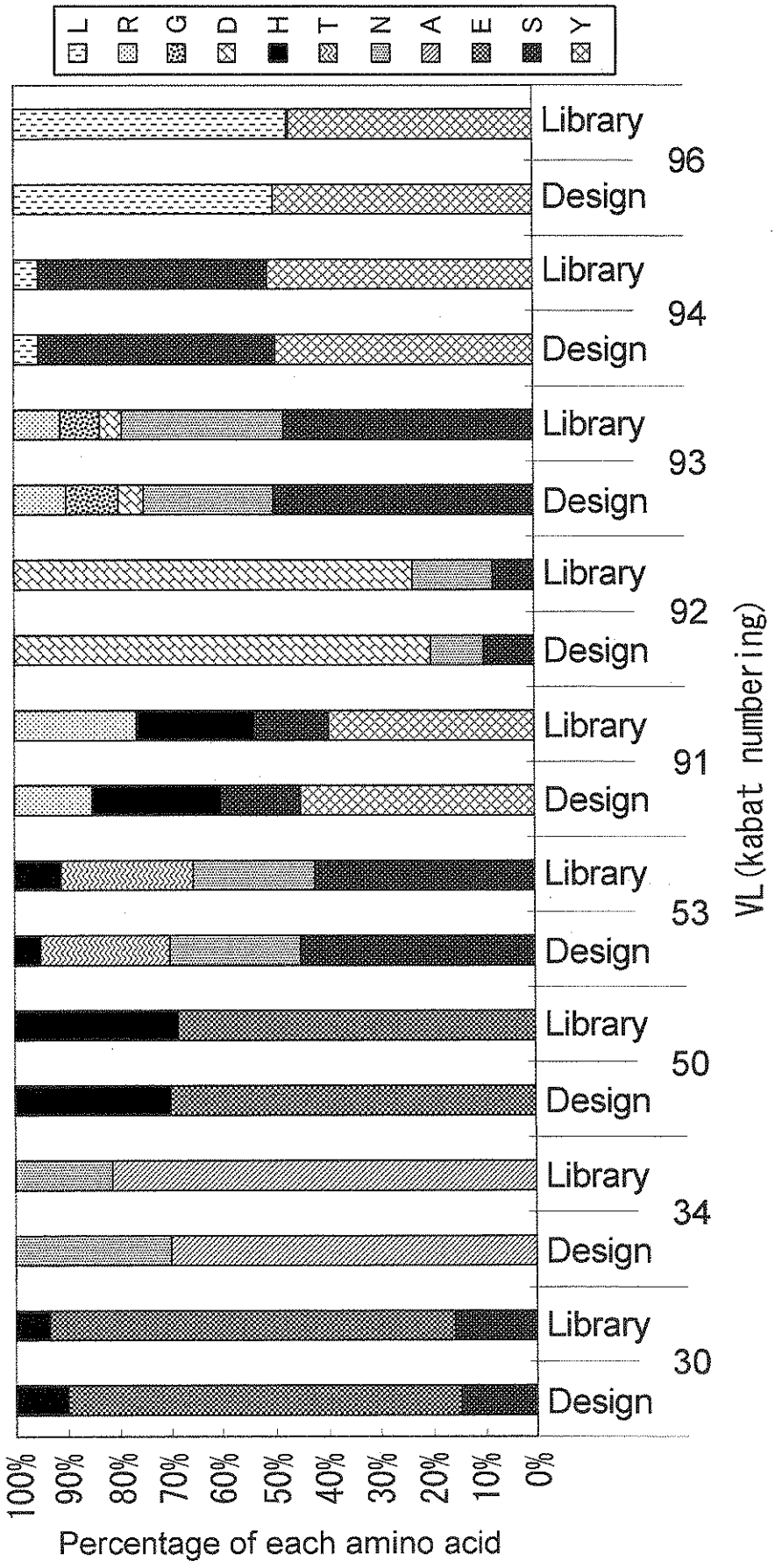

L
R
G
D
H
T
N
A
E
S
Y
Library
Design
96
94
93
92
91
53
50
34
30
VL (kabat numbering)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of each amino acid

FIG. 15

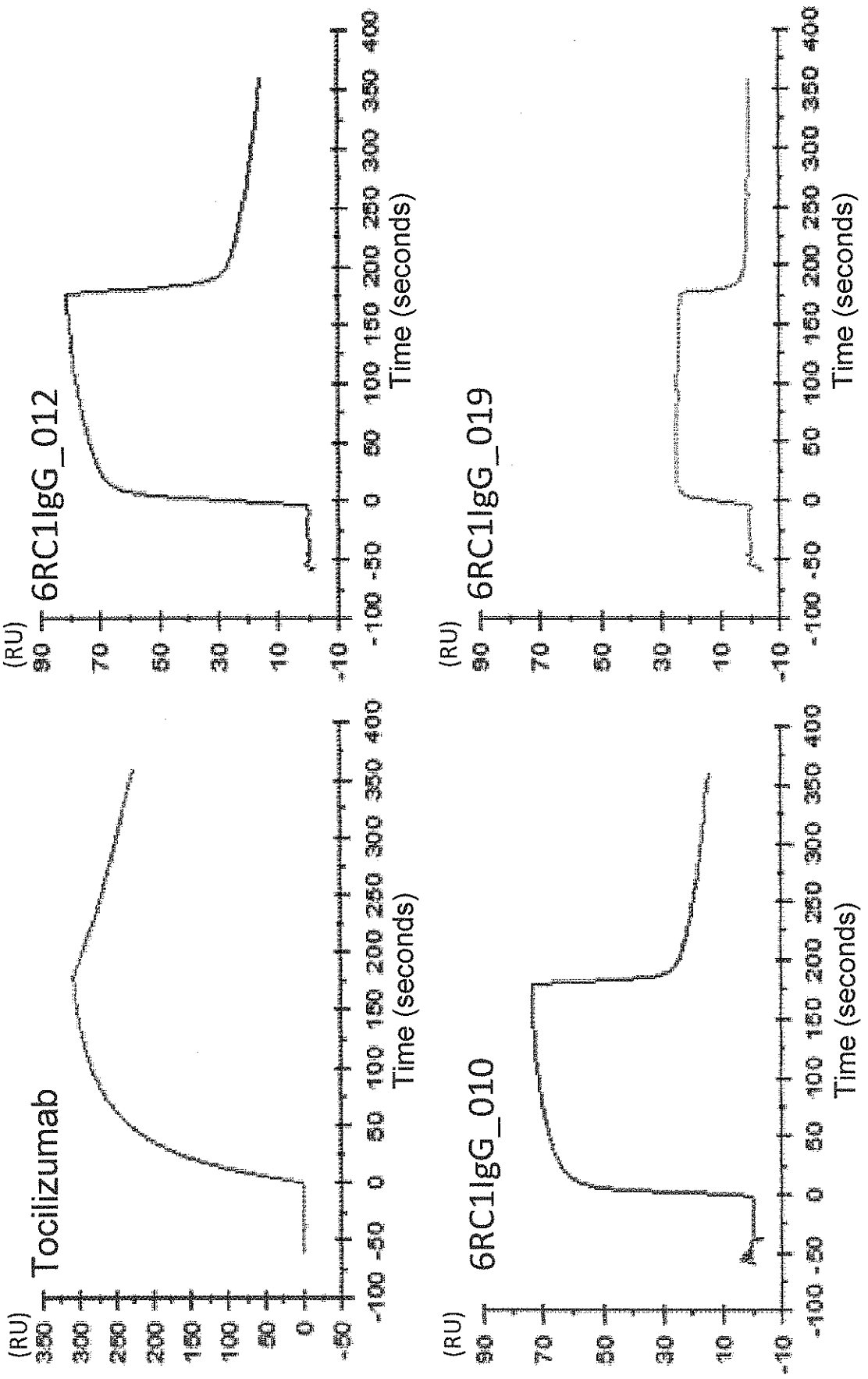

Tocilizumab
6RC1IgG_012
6RC1IgG_010
6RC1IgG_019
(RU)
Time (seconds)

FIG. 16

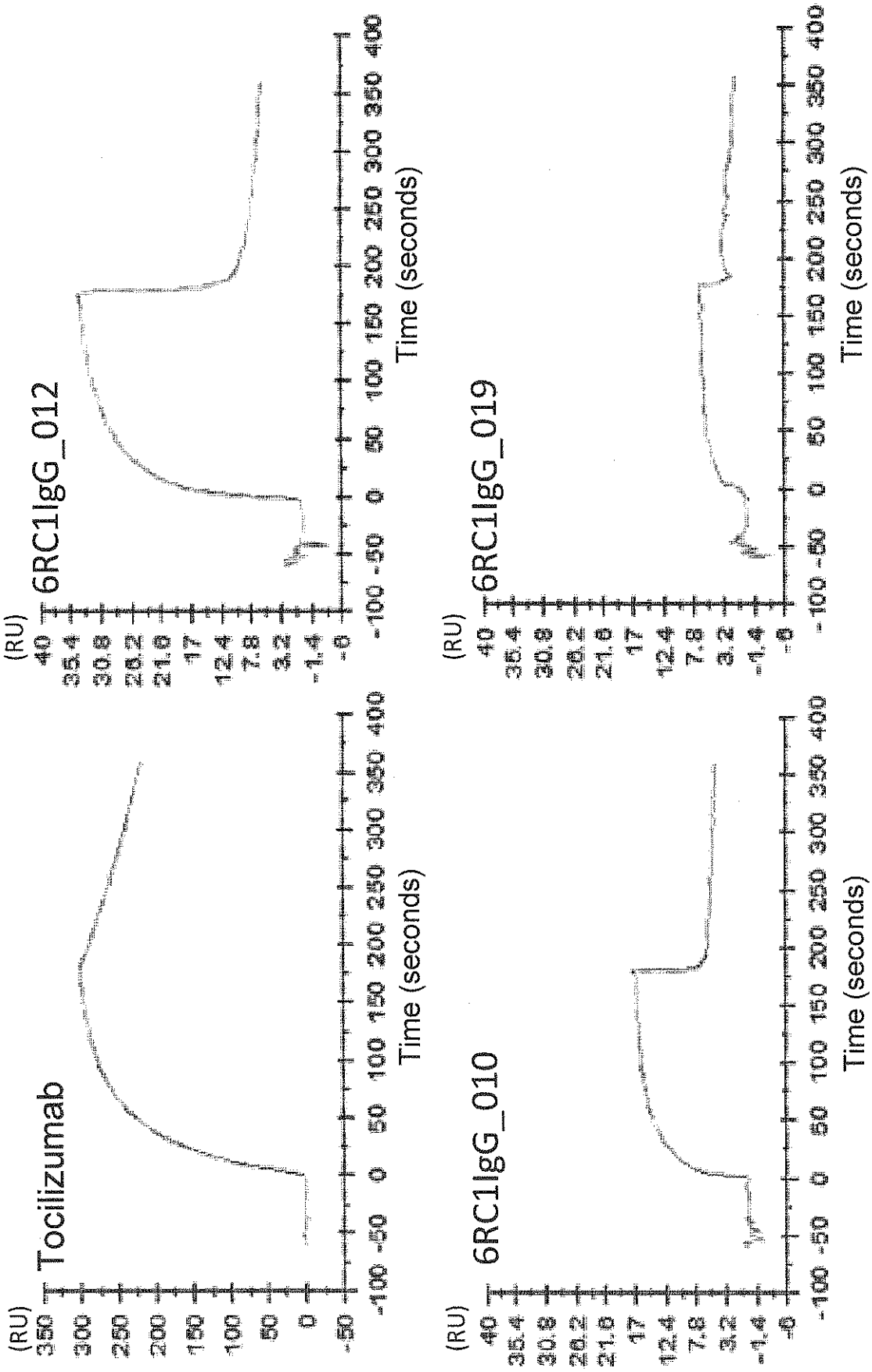
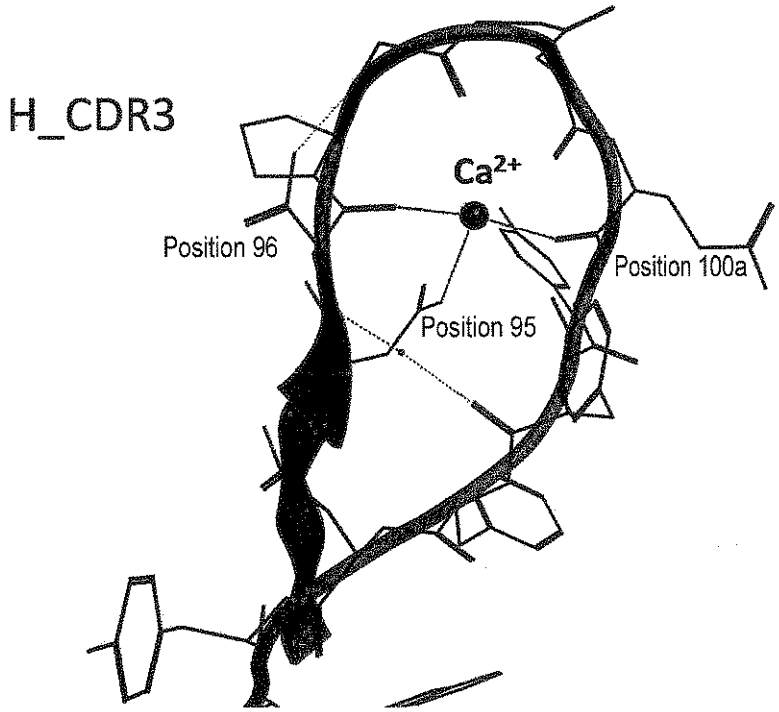

Tocilizumab
6RC1IgG_012
6RC1IgG_010
6RC1IgG_019
(RU)
Time (seconds)

FIG. 17

(i) Crystal structure in the presence of calcium ion



(ii) Crystal structure in the absence of calcium ion

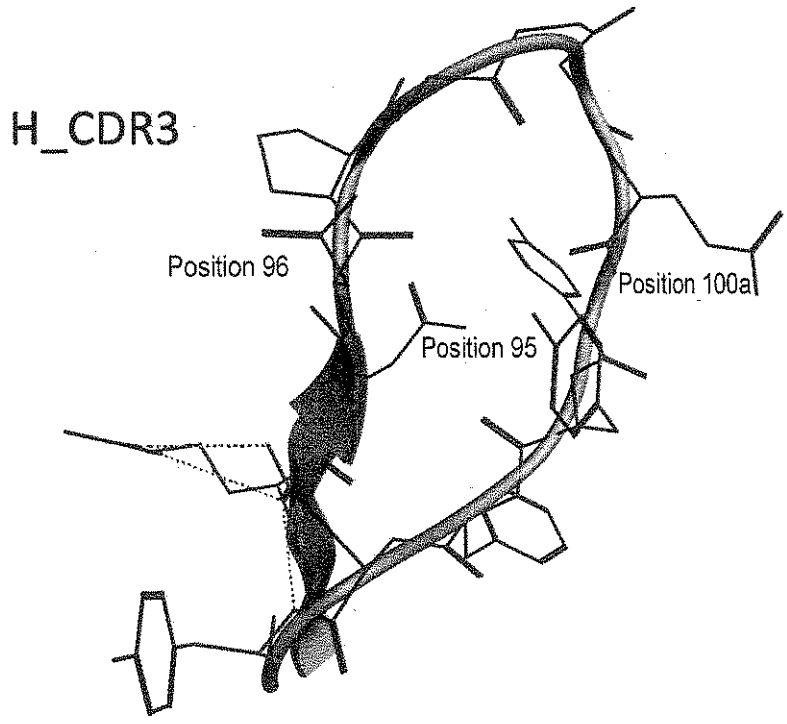


FIG. 18

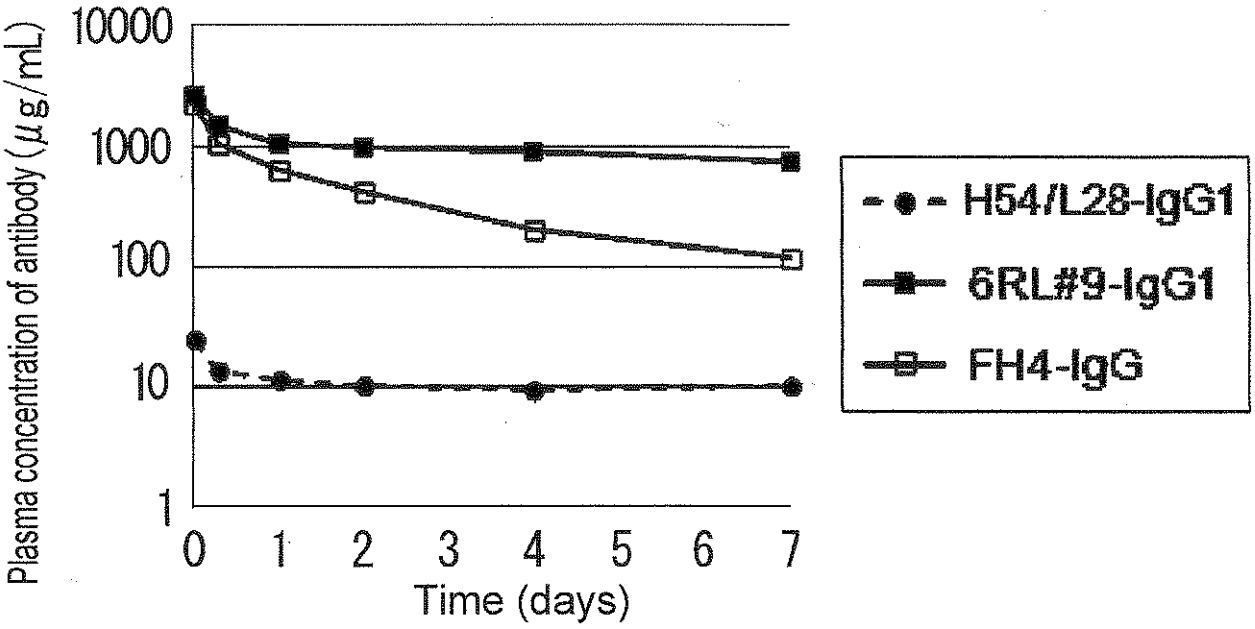

Plasma concentration of antibody (μg/mL)
10000
1000
100
10
1
0
1
2
3
4
5
6
7
Time (days)
H54/L28-IgG1
6RL#9-IgG1
FH4-IgG

FIG. 19

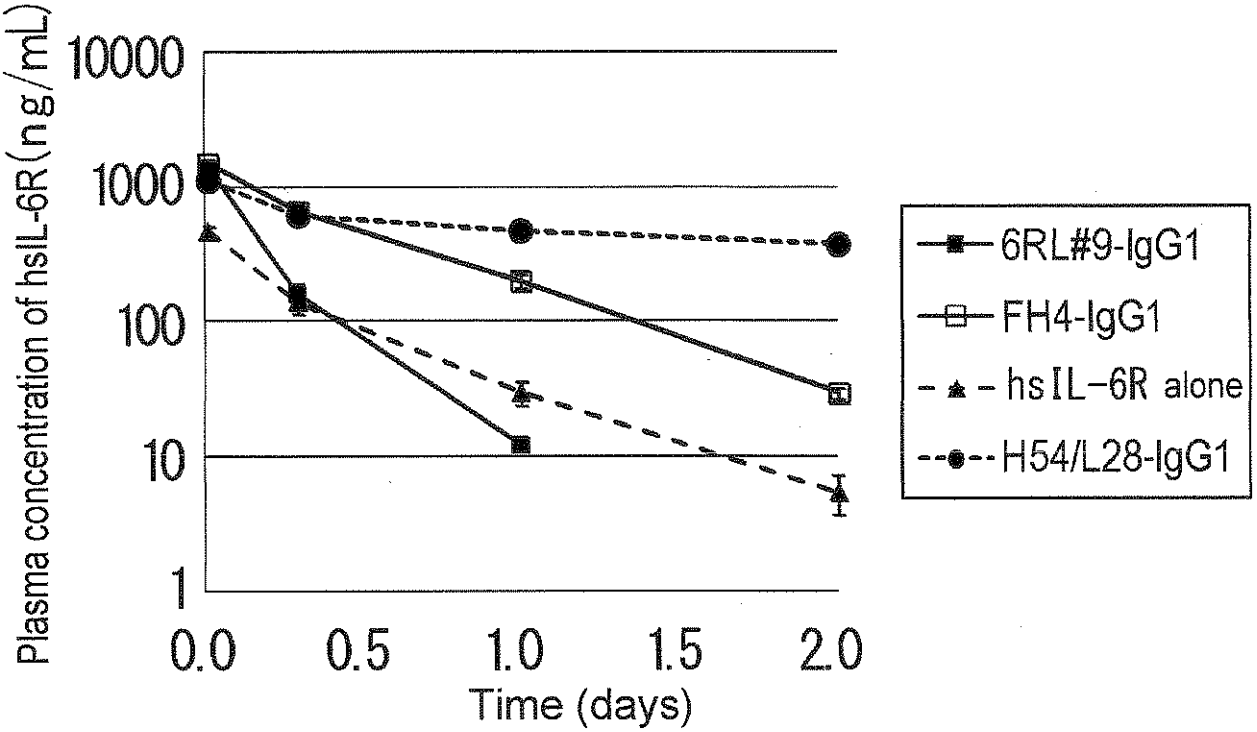

Plasma concentration of hsIL-6R(ng/mL)
10000
1000
100
10
1
0.0
0.5
1.0
1.5
2.0
Time (days)
6RL#9-IgG1
FH4-IgG1
hsIL-6R alone
H54/L28-IgG1

FIG. 20

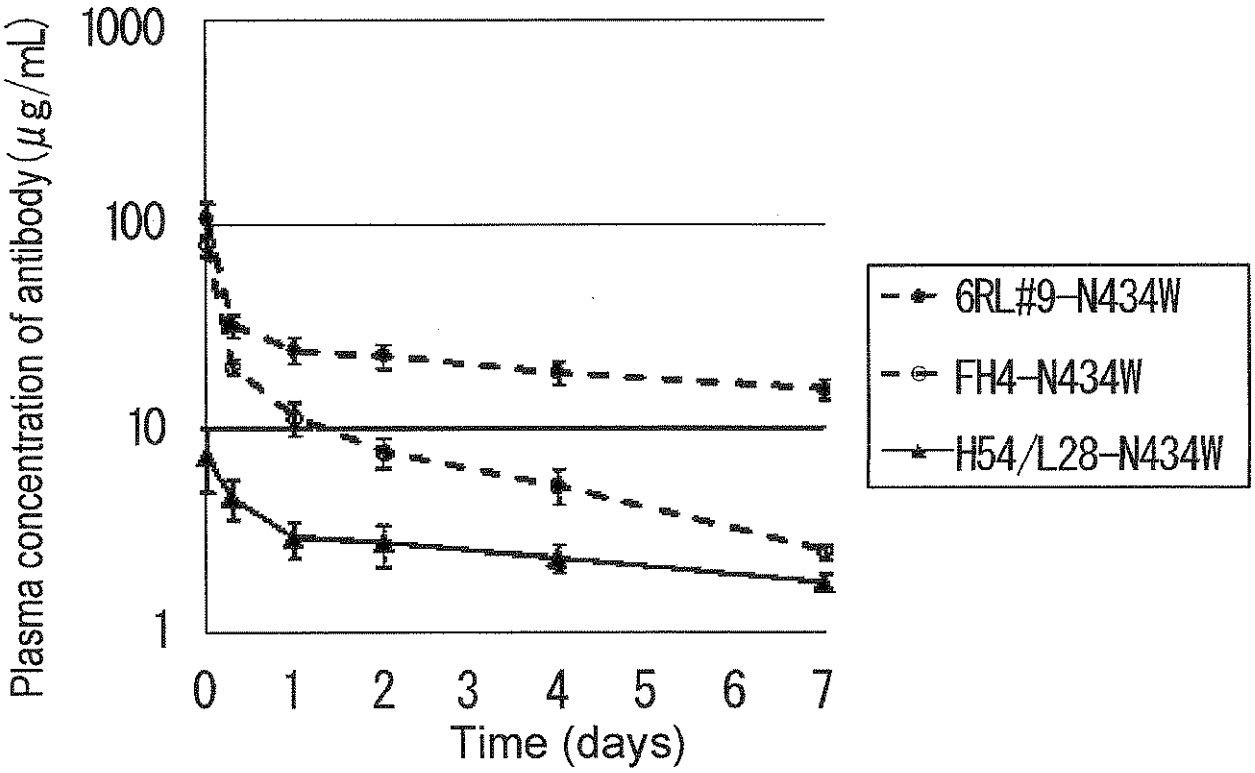

Plasma concentration of antibody (μg/mL)
1000
100
10
1
0
1
2
3
4
5
6
7
Time (days)
6RL#9-N434W
FH4-N434W
H54/L28-N434W

FIG. 21

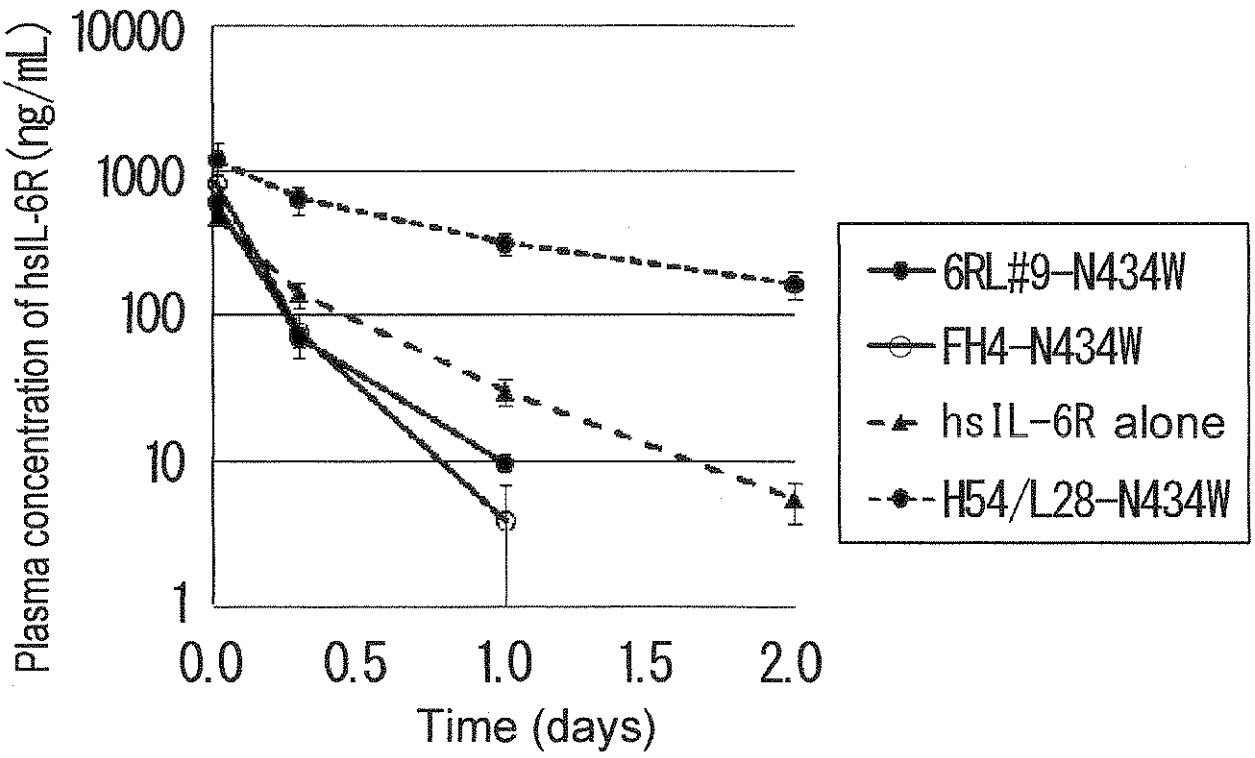

Plasma concentration of hsIL-6R (ng/mL)
10000
1000
100
10
1
0.0
0.5
1.0
1.5
2.0
Time (days)
6RL#9-N434W
FH4-N434W
hsIL-6R alone
H54/L28-N434W

FIG. 22

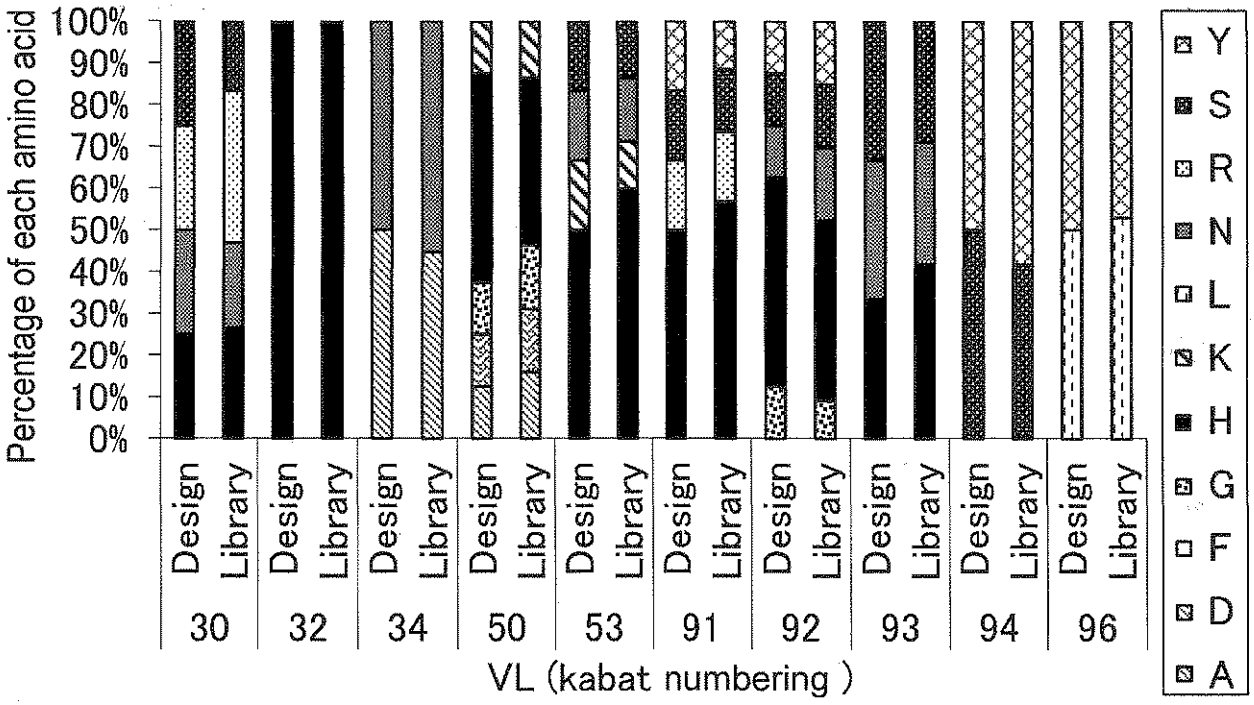

Percentage of each amino acid
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Design
Library
30
32
34
50
53
91
92
93
94
96
VL (kabat numbering)
Y
S
R
N
L
K
H
G
F
D
A

FIG. 23

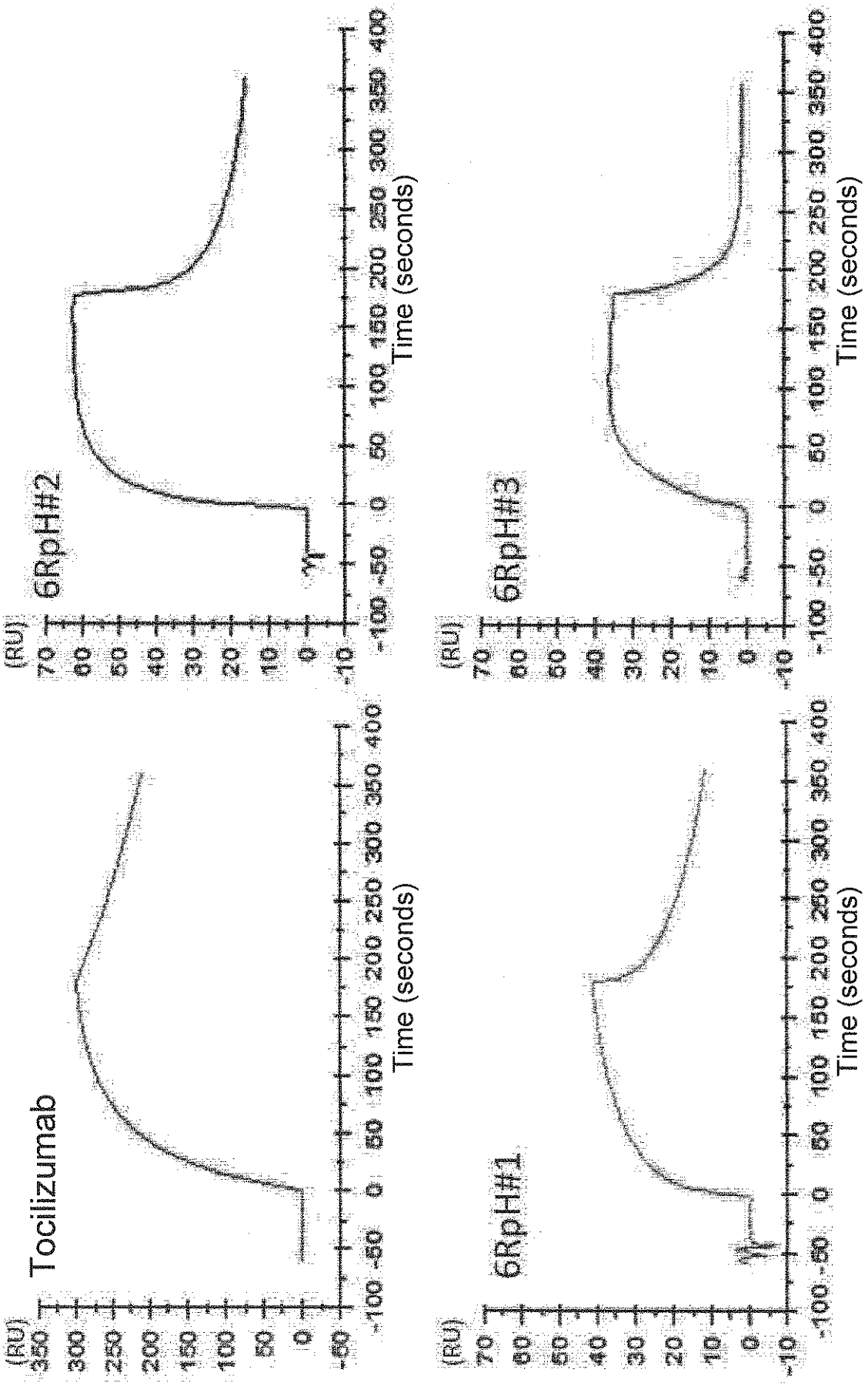


FIG. 24

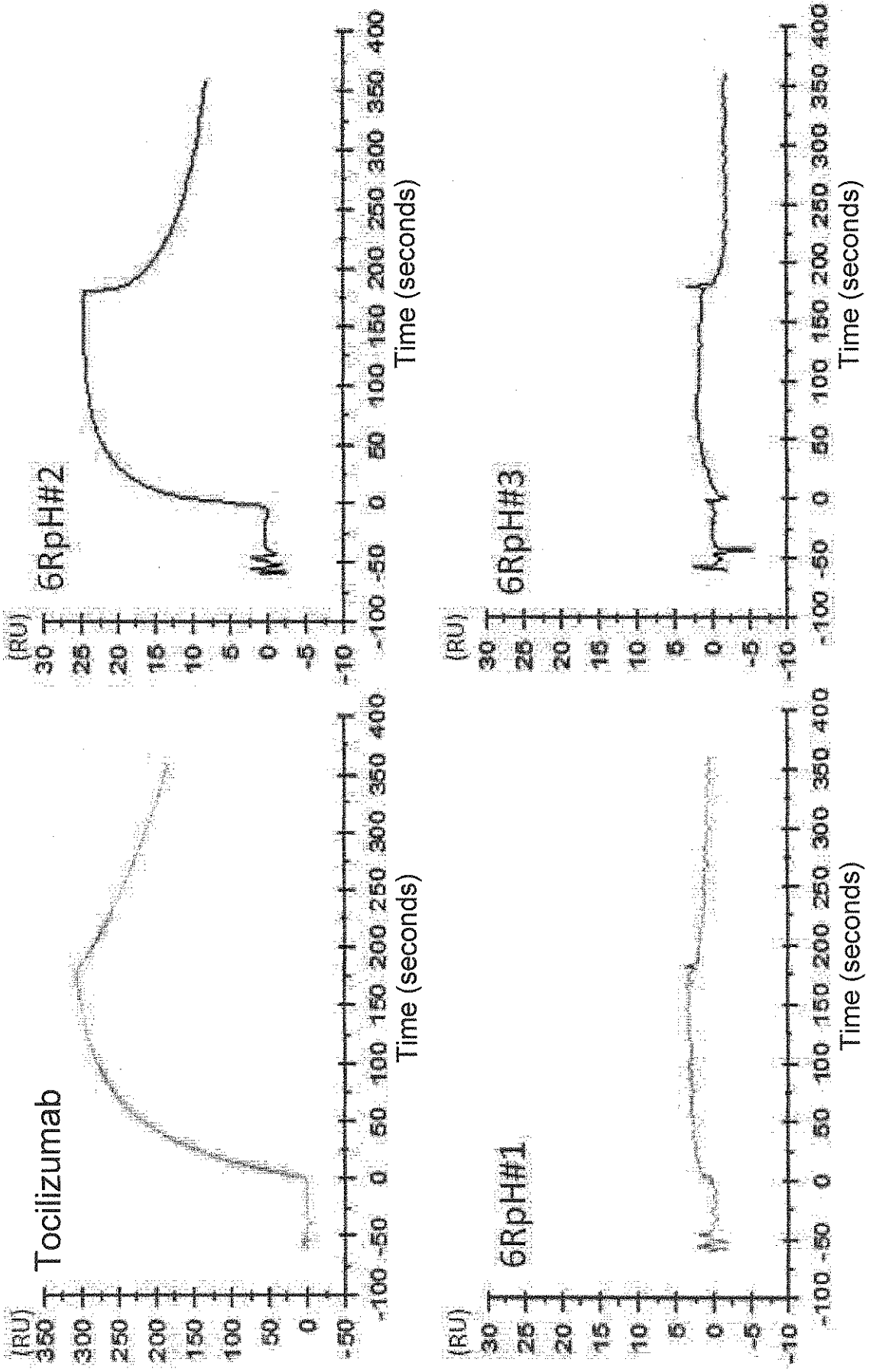


FIG. 25

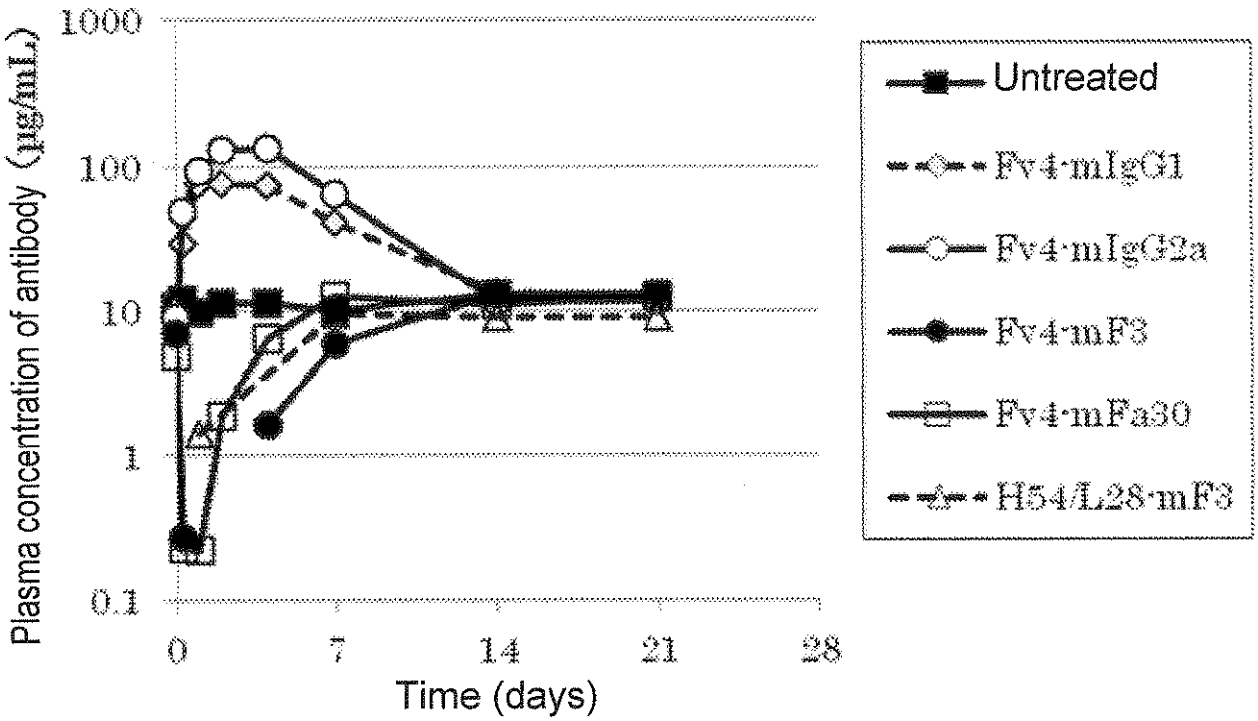

1000
100
10
1
0.1
Plasma concentration of antibody (μg/mL)
0
7
14
21
28
Time (days)
Untreated
Fv4-mIgG1
Fv4-mIgG2a
Fv4-mF3
Fv4-mFa30
H54/L28-mF3

FIG. 26

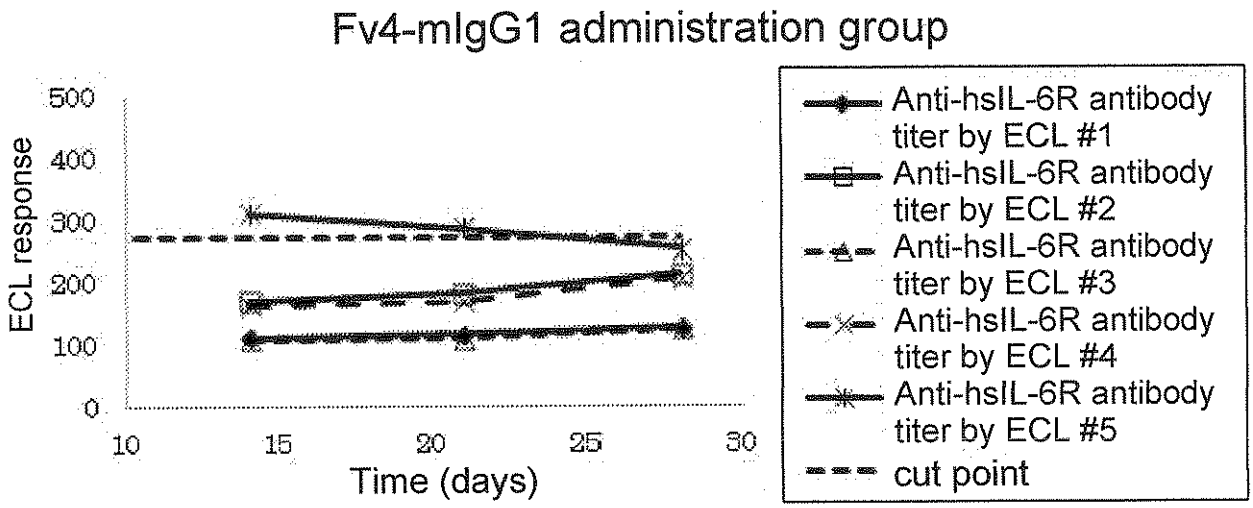

Fv4-mIgG1 administration group
ECL response
500
400
300
200
100
0
10
15
20
25
30
Time (days)
Anti-hsIL-6R antibody titer by ECL #1
Anti-hsIL-6R antibody titer by ECL #2
Anti-hsIL-6R antibody titer by ECL #3
Anti-hsIL-6R antibody titer by ECL #4
Anti-hsIL-6R antibody titer by ECL #5
cut point

FIG. 27

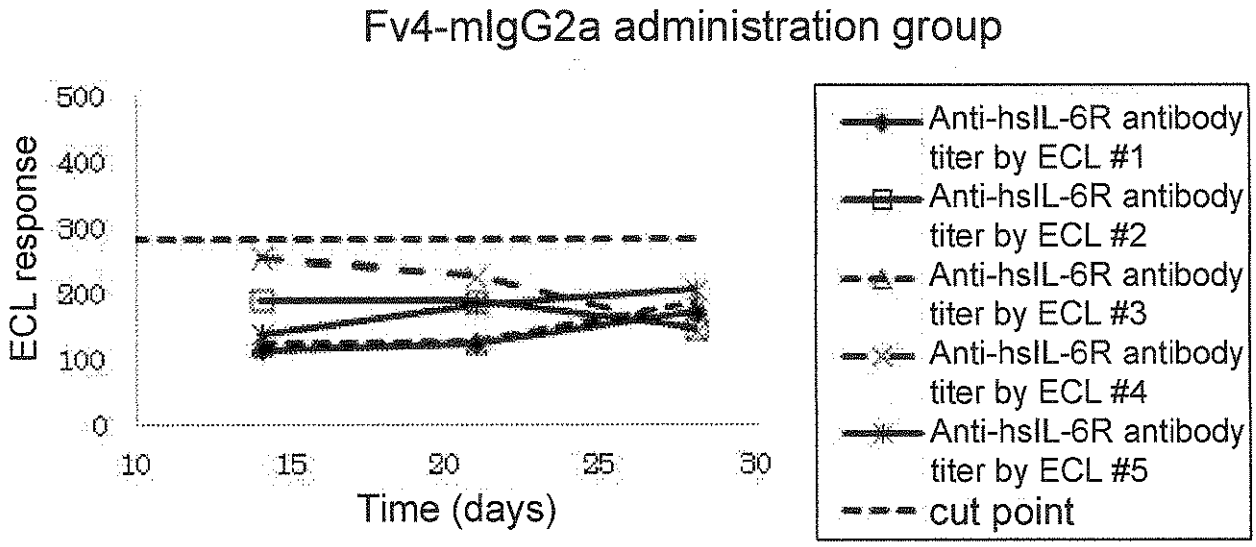

Fv4-mIgG2a administration group
ECL response
500
400
300
200
100
0
10
15
20
25
30
Time (days)
Anti-hsIL-6R antibody titer by ECL #1
Anti-hsIL-6R antibody titer by ECL #2
Anti-hsIL-6R antibody titer by ECL #3
Anti-hsIL-6R antibody titer by ECL #4
Anti-hsIL-6R antibody titer by ECL #5
cut point

FIG. 28

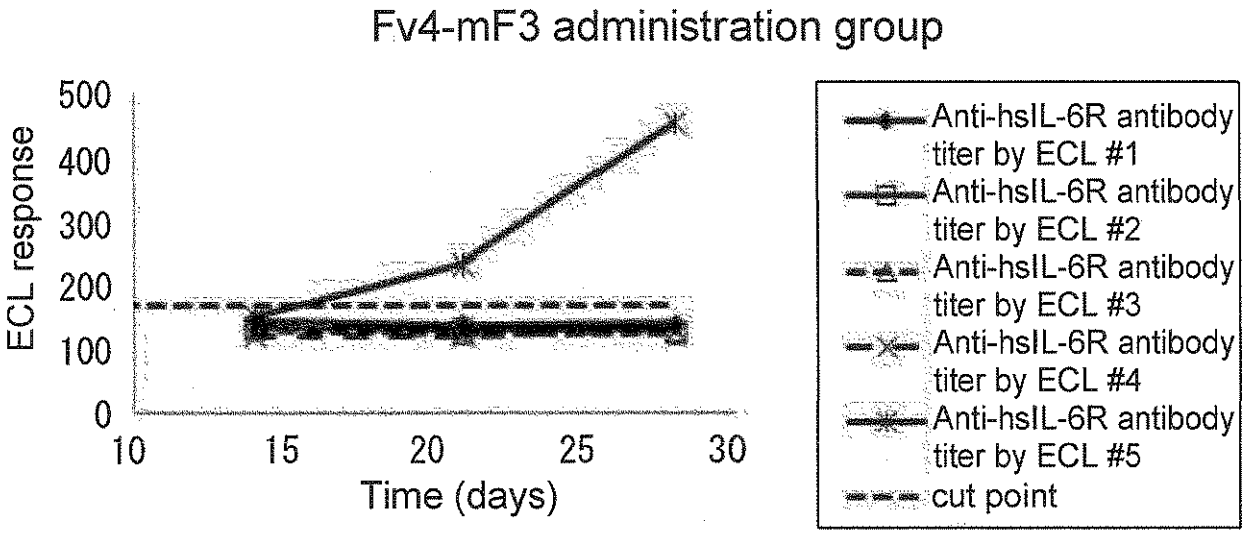

Fv4-mF3 administration group
ECL response
500
400
300
200
100
0
10
15
20
25
30
Time (days)
Anti-hsIL-6R antibody titer by ECL #1
Anti-hsIL-6R antibody titer by ECL #2
Anti-hsIL-6R antibody titer by ECL #3
Anti-hsIL-6R antibody titer by ECL #4
Anti-hsIL-6R antibody titer by ECL #5
cut point

FIG. 29

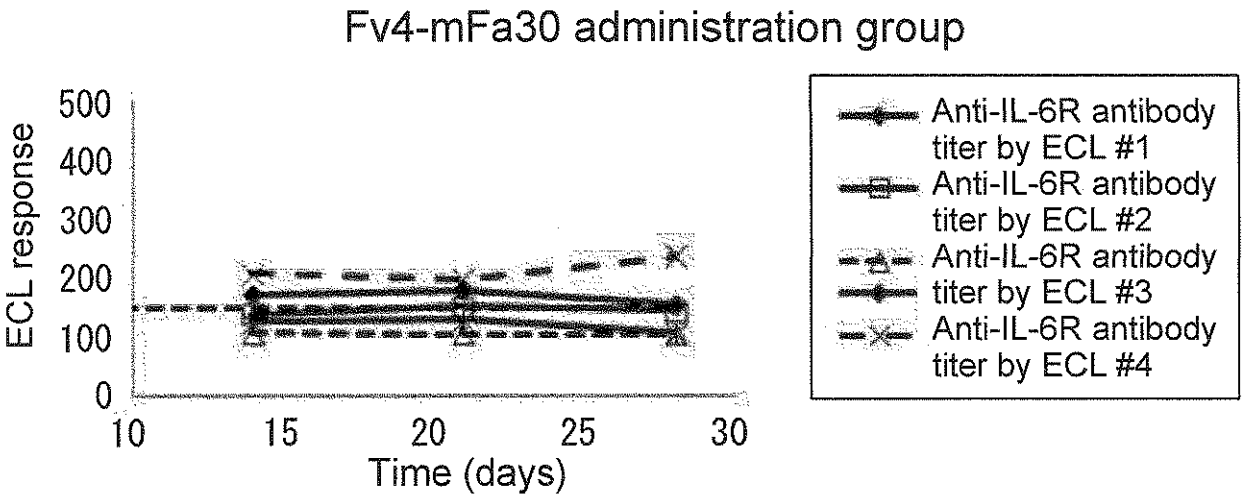

Fv4-mFa30 administration group
ECL response
500
400
300
200
100
0
10
15
20
25
30
Time (days)
Anti-IL-6R antibody titer by ECL #1
Anti-IL-6R antibody titer by ECL #2
Anti-IL-6R antibody titer by ECL #3
Anti-IL-6R antibody titer by ECL #4

FIG. 30

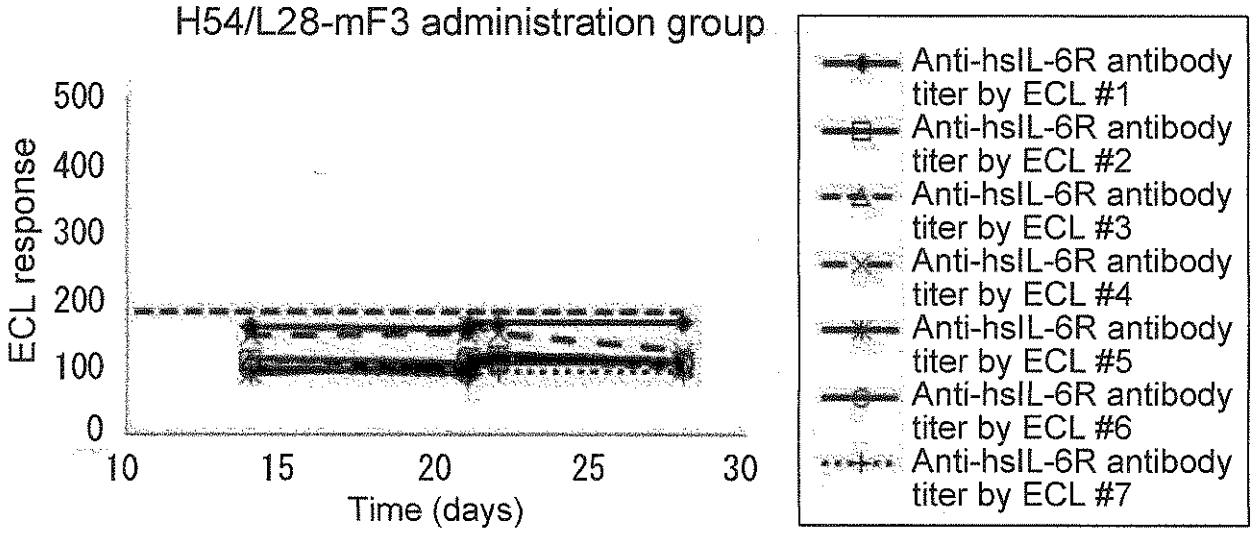

H54/L28-mF3 administration group
ECL response
500
400
300
200
100
0
10
15
20
25
30
Time (days)
Anti-hsIL-6R antibody titer by ECL #1
Anti-hsIL-6R antibody titer by ECL #2
Anti-hsIL-6R antibody titer by ECL #3
Anti-hsIL-6R antibody titer by ECL #4
Anti-hsIL-6R antibody titer by ECL #5
Anti-hsIL-6R antibody titer by ECL #6
Anti-hsIL-6R antibody titer by ECL #7

FIG. 31

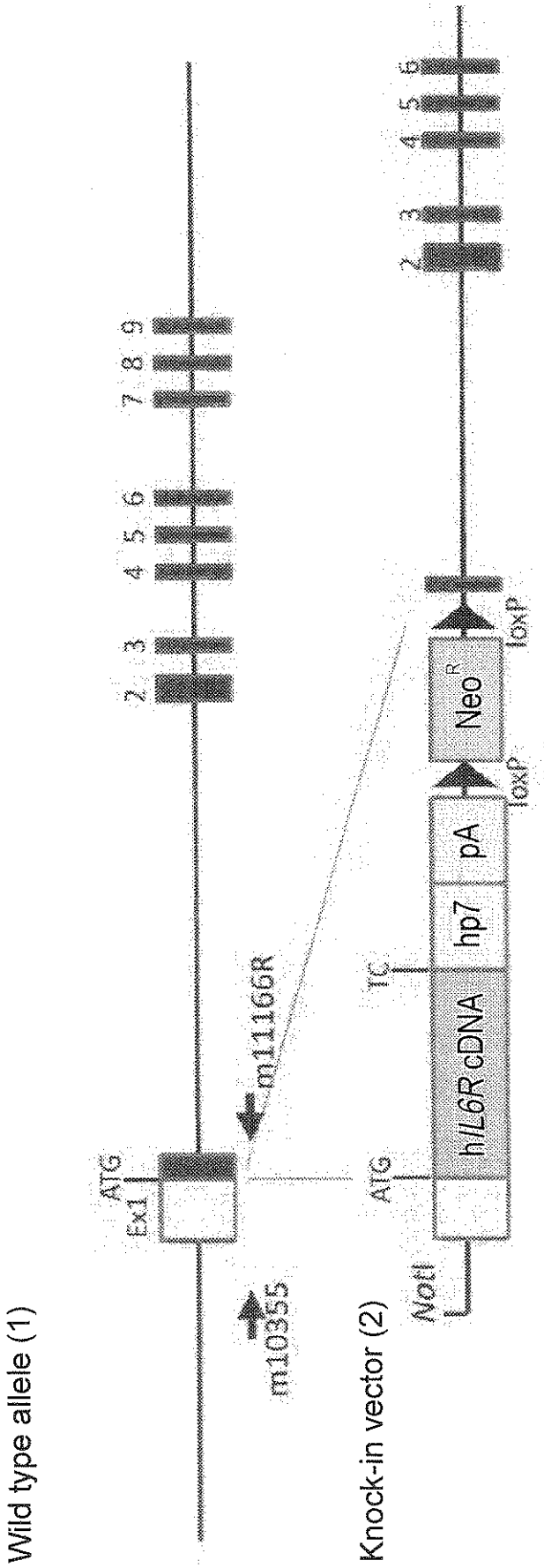

Wild type allele (1)
ATG
Ex1
m11166R
m10355
2 3 4 5 6 7 8 9
Knock-in vector (2)
ATG
TC
h/L6R cDNA
hp7
pA
loxP
Neo^R
loxP
NotI
2 3 4 5 6

FIG. 32A

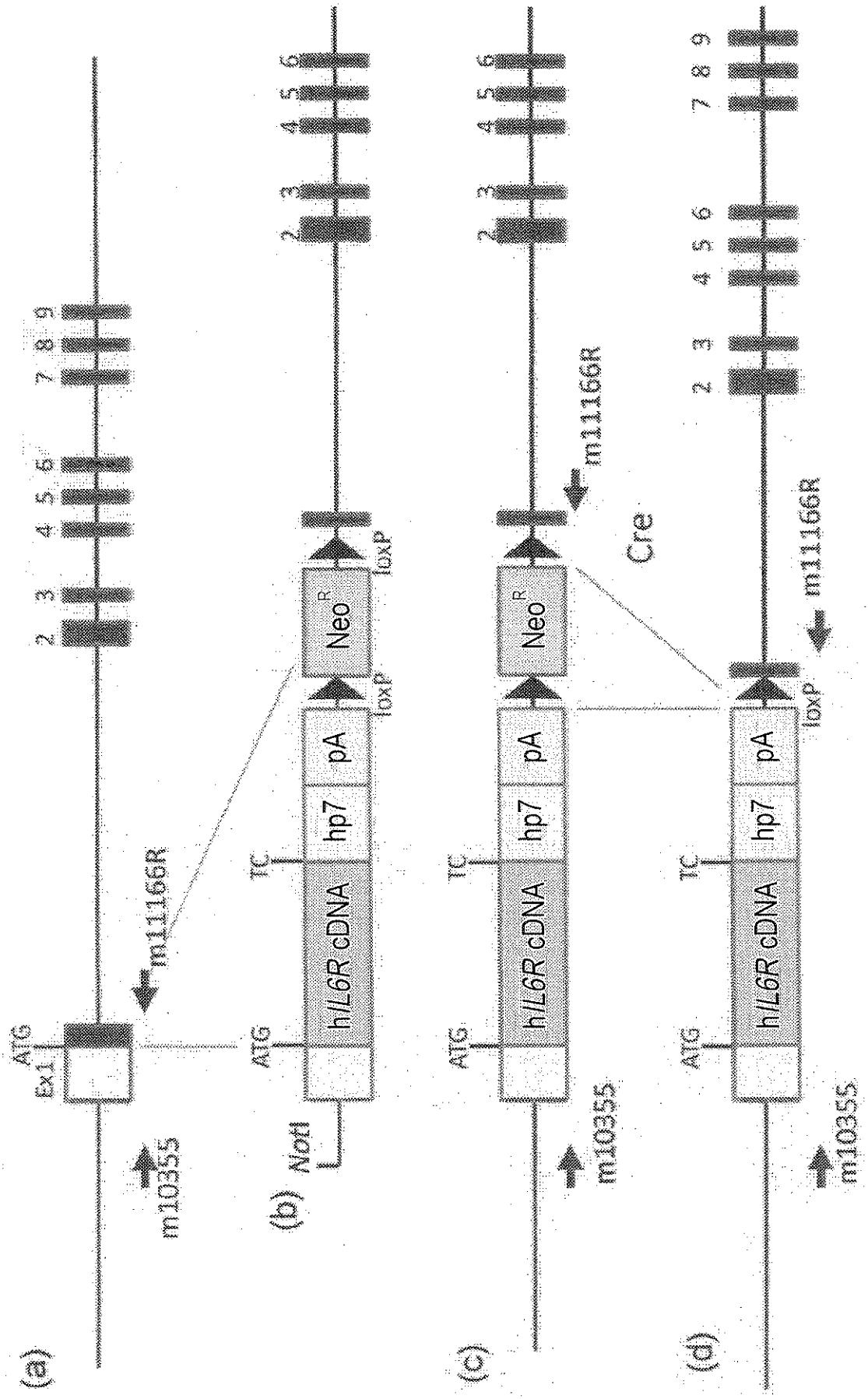

(a)
ATG
Ex1
m11166R
m10355
2 3 4 5 6 7 8 9
(b)
NotI
ATG
h/L6R cDNA
hp7
pA
loxP
Neo^R
loxP
TC
2 3 4 5 6
(c)
m11166R
Cre
(d)
m11166R
2 3 4 5 6 7 8 9

FIG. 32B

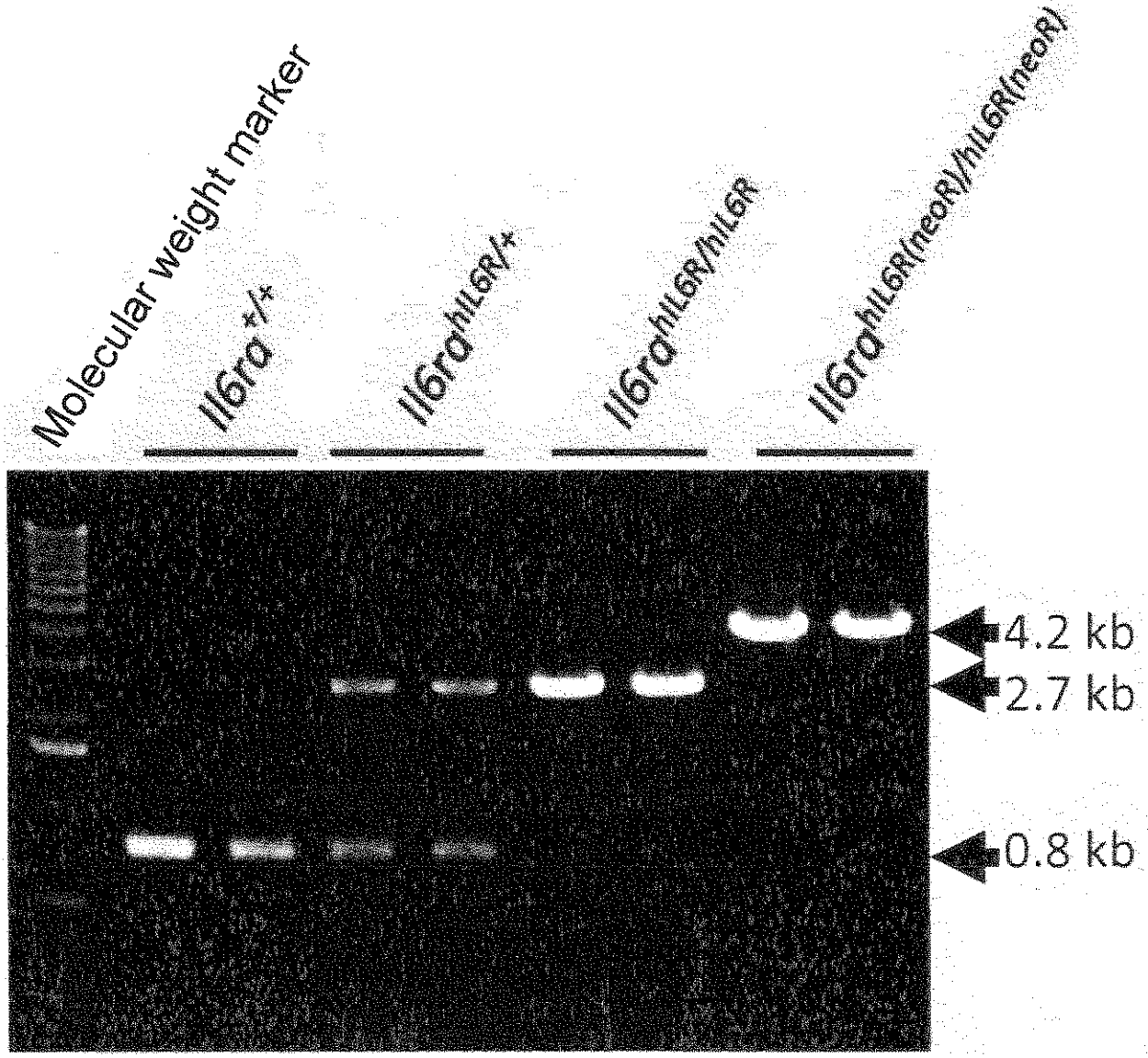

Molecular weight marker
Il6ra +/+
Il6ra hIL6R/+
Il6ra hIL6R/hIL6R
Il6ra hIL6R(neoR)/hIL6R(neoR)
4.2 kb
2.7 kb
0.8 kb

FIG. 33

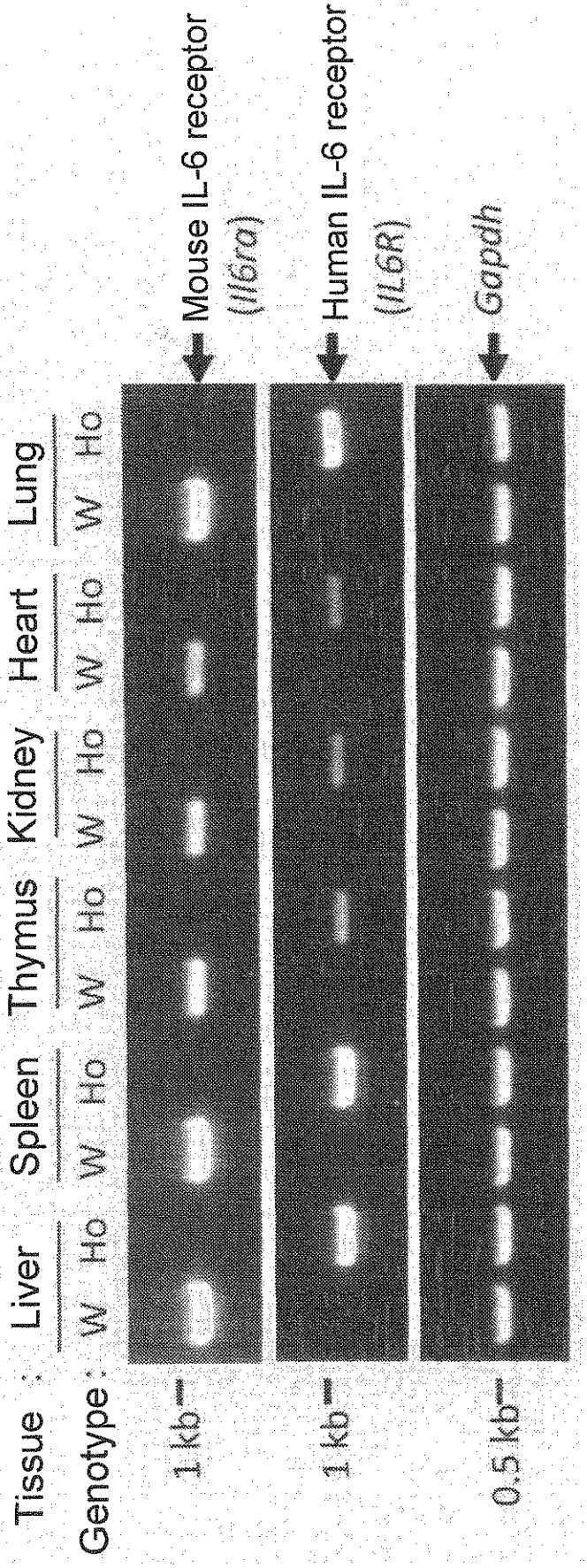

Tissue : Liver Spleen Thymus Kidney Heart Lung
Genotype : W Ho W Ho W Ho W Ho W Ho W Ho
Mouse IL-6 receptor
(Il6ra)
Human IL-6 receptor
(IL6R)
Gapdh
1 kb
1 kb
0.5 kb

FIG. 34

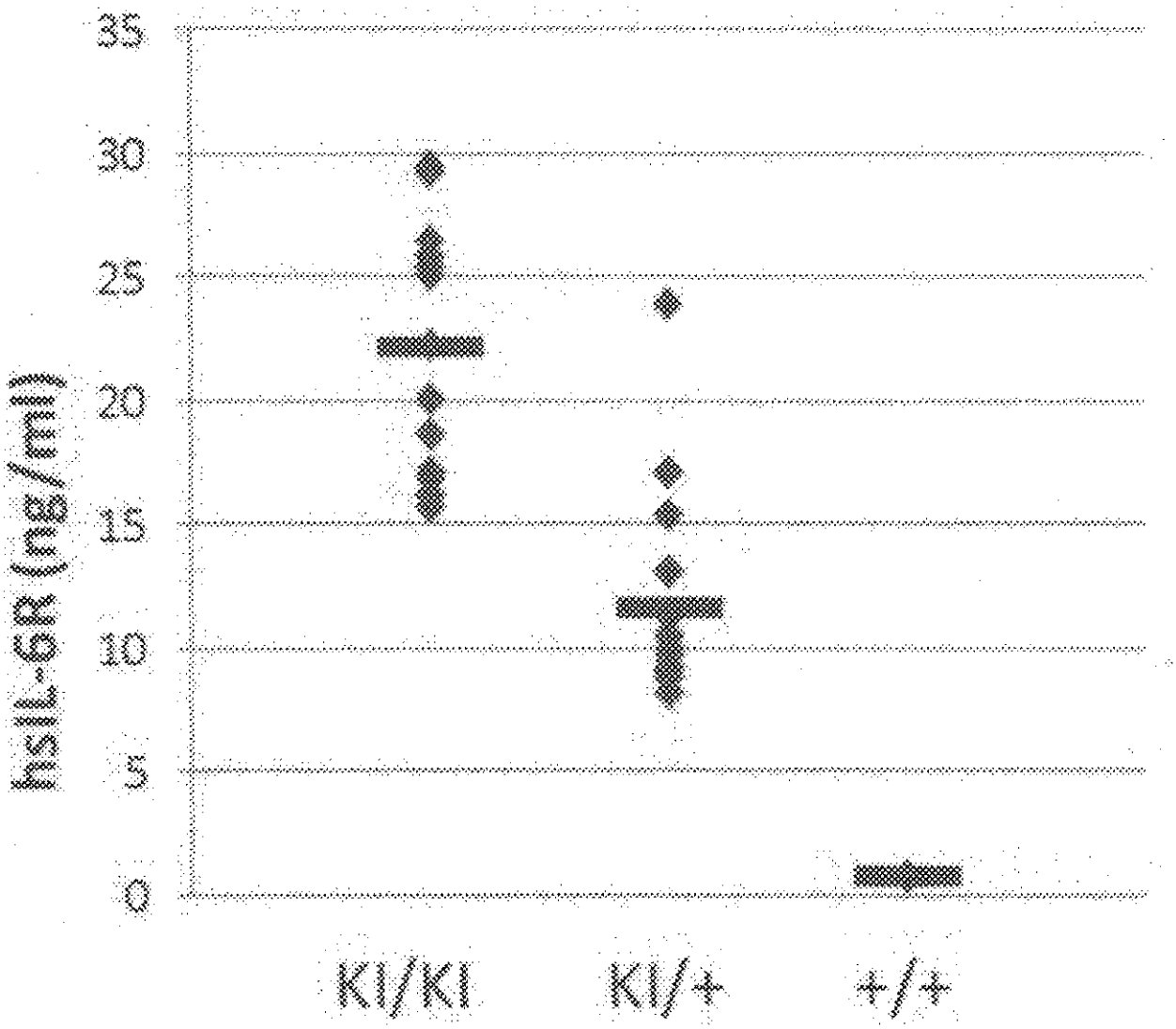


FIG. 35

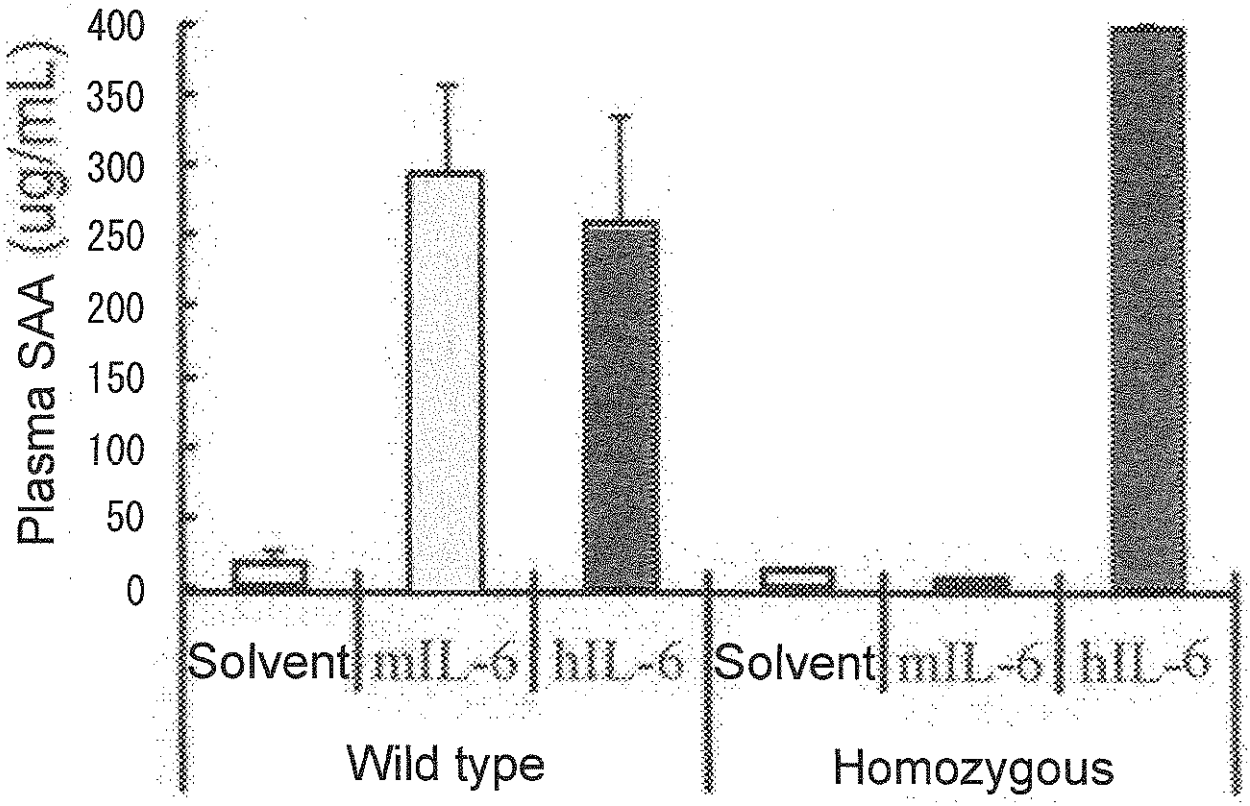

Plasma SAA (ug/mL)
400
350
300
250
200
150
100
50
0
Solvent
mIL-6
hIL-6
Solvent
mIL-6
hIL-6
Wild type
Homozygous

FIG. 36